Dipicolinic acid with metal (II) cations as a nutrient source for soil bacteria

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Abstract

Hydraulic fracturing is an efficient way to recover natural gas from rock formations beneath the surface of the ground. However, it involves the injection of highly pressurized water containing chemicals including heavy metal cations into the ground, producing millions of gallons of hazardous wastewater that cannot be reused. It has been suggested that pyridine-2,6-dicarboxylic acid, also known as dipicolinic acid (DPA), which is produced by the spores of *Bacillus subtilis*, is able to chelate to the metal cations that are present in the hydrofracking wastewater.[1] However, the *Bacillus subtilis* itself is unable to utilize the DPA as a nutrient source. This work focuses on the ability of other soil bacteria to utilize DPA and these metals as a nutrient source, with major interest on the question of whether these bacteria are metabolizing and eliminating these complexes from the water, therefore effectively working towards the bioremediation of the wastewater from hydraulic fracturing.

Introduction

Large volumes of wastewater are produced as a result of hydraulic fracturing, and this water contains very high concentrations of dissolved solids, which includes the heavy metal cations studied in this work.[2] The metals of particular interest in this research include barium, calcium, magnesium, and strontium. Currently, the major focus is on strontium due to its radioactivity and resulting hazard to humans, causing serious health problems such as cancer.[3] Finding a successful bioremediation method for this wastewater in order to remove these harmful metals is essential for hydraulic fracturing to be used safely as a way to recover natural gas as a source of energy.

Methods

Agar plates were prepared, containing noble agar (1.5%), minimal media – ammonium sulfate (0.2%), dipotassium phosphate (1.4%), monopotassium phosphate (0.6%), sodium chloride (0.1%), and magnesium chloride (0.02%) – and variable metals. The minimal media contained all necessary nutrients required for bacteria to grow except for a carbon source, which was provided by either glucose or DPA. Bacteria were streaked from an original plate culture onto experimental plates. Growth was observed over a period of one week.

Results

Table 1: Results of bacterial growth on noble agar/minimal media plates containing glucose (control) or variable glucose or DPA and metal combinations.

Minimal Media Plate Components	Relative Amount of Growth
Glucose only	++
DPA only	0
Glucose + $CaCl_2$	++++
DPA + $CaCl_2$	++++
Glucose + $SrCl_2$	0
DPA + $SrCl_2$	++++
Glucose + DPA	0

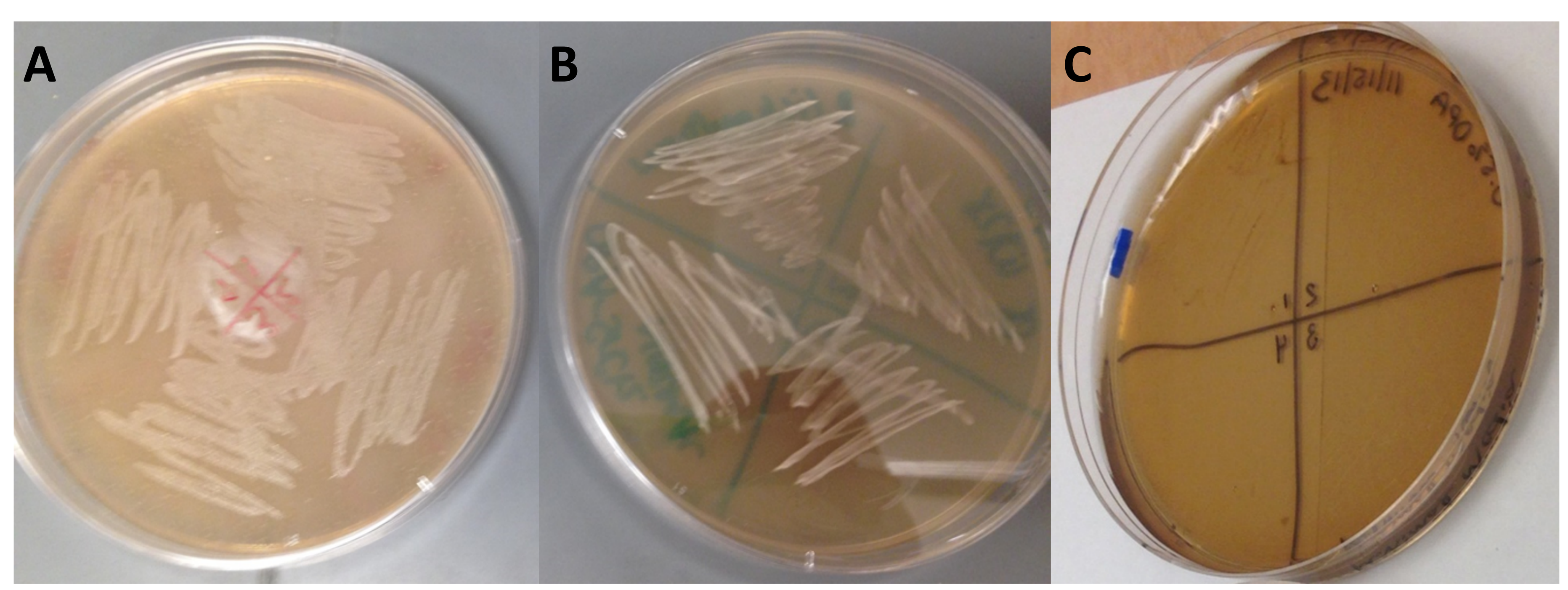

Figure 1: Noble agar/minimal media plates containing the following: (A) glucose and $CaCl_2$ in a 1:2 ratio; (B) DPA and $SrCl_2$ in a 1:2 ratio; (C) DPA only.

References

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2. Gregory, K.B.; Vidic, R.D.; Dzombak, D.A. Water Management Challenges Associated with the Production of Shale Gas by Hydraulic Fracturing. *Elements*. **2011**, *7*(3), 181-186.
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Discussion

Bacterial growth in the presence of DPA and heavy metal cations was studied. It is known from previous research that the bacteria will grow in the presence of glucose only, glucose and $CaCl_2$ in a 1:2 molar ratio, and DPA and $CaCl_2$ in a 1:2 molar ratio. The bacteria will not grow, however, in the presence of DPA only or in the presence of DPA and glucose together in a 1:1 molar ratio. It was discovered that the bacteria grow when DPA and $SrCl_2$ are present, but not when glucose and $SrCl_2$ are present. This suggests that when DPA and strontium are complexed, the toxicity of each is eliminated and the bacteria are able to utilize the complex as a carbon source. These results imply that this soil bacteria may be used to bioremediate wastewater by eliminating some of the toxic heavy metals present in the water after the retrieval of natural gas.

Future Directions

- Monitor growth of bacteria in the presence of DPA and heavy metals using liquid bacterial cultures.
- Use Flame Atomic Absorption to determine the amounts of heavy metals removed from liquid cultures.