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# Desert soil bacteria deposit atmospheric carbon dioxide in carbonate precipitates

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ABSTRACT

Carbonate precipitation by soil microbes has generated major concern recently because of the potential for mitigating the challenge of increasing $CO_2$ level; however, while desert soil microbes have the capacity of carbonate formation, it is unknown if they can deposit atmospheric carbon dioxide. We isolated soil bacteria from the Mu Us Desert in northern China to perform an experiment of carbonate formation by bacteria isolated from the soil. We detected the precipitates produced by the bacteria, and identified the carbon source of the carbonate precipitates using stable carbon isotopic tracing. Six bacterial strains, designated as strains 1–6, were isolated from the desert soil, including *Arthrobacter* sp., *Rhodococcus* sp., *Planococcus* sp., *Streptomyces* sp., *Arthrobacter* sp., and *Microbacterium* sp. We confirmed that all six species precipitated carbonate (vaterite and calcite) using X-ray diffraction, scanning electron microscopy, and energy dispersive X-ray. $^{13}CO_2$ labeling showed that the abundance of $^{13}C$ was significantly higher in carbonate precipitates, of which 1.03% was represented by atmospheric carbon; that is, the atmospheric $CO_2$ participates in the formation of carbonate by desert soil bacteria. To our knowledge, this is the first study to demonstrate the occurrence of carbonate precipitation by these desert soil bacterial strains (strains 1, 2, 4, 5, and 6), which expands the current knowledge on carbonate-depositing bacteria in drylands. The carbon transformation process, from atmospheric $CO_2$ to carbonate precipitates by soil bacteria, indicates a process of atmospheric $CO_2$ deposition through microbial carbonate precipitation. Although under controlled conditions, this study provided an important insight supporting that drylands probably contribute strongly to global carbon processes via soil microbial activity.

## 1. Introduction

The increase in atmospheric carbon dioxide ($CO_2$) through anthropogenic activities is considered as a cause for global warming, with a consequent significant influence on the Earth's climate (Drake, 2000). In view of this, there has been increasing focus on eliminating or preventing increases in $CO_2$ levels through various ways, such as $CO_2$ sequestration by plants, cyanobacteria, algae, and physicochemical processes (Cannon et al., 2001; Gilfillan et al., 2009; Mitchell et al., 2010; Matter et al., 2016; Wang et al., 2016; Abid et al., 2017; Hicks et al., 2017). Through these processes, atmospheric $CO_2$ could be sequestrated in the soil, ocean, basalt, and deep-sea aquifers. Some studies have reported the processes of carbonate biomineralization by microbes; that is, some microbes are capable of precipitating $CO_2$ and/or dissolved inorganic carbon (DIC, including $HCO_3^-$ and $CO_3^{2-}$) as stable carbonate forms, such as calcite and vaterite (Hammes and Verstraete, 2002; Bosak, 2011; Rodriguez-Navarro et al., 2012). This carbon biomineralization process is considered as one of the most effective methods to mitigate the challenge of increasing $CO_2$ levels (Srivastava et al., 2015; Okyay et al., 2016), and hence, is of great interest. Because drylands are widespread in terrestrial ecosystems (Lal, 2009), the potential of carbonate precipitation by microbes in these areas might be important in sequestrating atmospheric $CO_2$ (Mitchell et al., 2010; Srivastava et al., 2015). Thus, an understanding of atmospheric $CO_2$ deposition via microbial carbonate precipitation in drylands is of importance for atmospheric $CO_2$ sequestration.

Carbonate precipitation by soil microbes in drylands has been increasingly investigated over the last twenty years (Monger et al., 1991; Danin et al., 1998; Delgado et al., 2008; Durand et al., 2010; Khormali et al., 2014; Wang et al., 2016; Zamanian et al., 2016). Microbe-originated carbonate precipitates, such as nanobacteria-like calcite, needle-like calcite, and spherulites, have been found in the South Tunisia Desert, semiarid areas of Israel, and caliches of west Texas (USA), and are most likely the products of bacterial metabolic activities

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(Verrecchia, 1990; Benzerara et al., 2003; Zhou and Chafetz, 2009). Carbonate precipitates could be formed in a short term in soils by microbes with the addition of Ca-rich solution in the Chihuahuan Desert (USA) (Monger et al., 1991). The processes of calcium carbonate ($CaCO_3$) precipitate formation by microbes have also been determined in culture media with bacteria isolated from semi-arid saline soil (Delgado et al., 2008), and with the indigenous microbial populations obtained from the Chihuahuan Desert (USA) (Monger et al., 1991; Khormali et al., 2014; Wang et al., 2016). These multiple lines of evidence suggest that soil microbes in drylands precipitate carbonate. However, where carbon comes from and whether atmospheric $CO_2$ can be deposited via this biomineralization process remain unknown, representing an important gap in the knowledge of carbon sequestration via microbial carbonate deposition.

In this study, we hypothesized that there are some soil microbes in the Mu Us Desert of northern China that precipitate carbonate, and deposit atmospheric $CO_2$ in carbonate precipitates through this process. To test this hypothesis, we isolated soil bacteria from the Mu Us Desert in northern China to perform an experiment of carbonate formation by bacteria in the laboratory. Within this framework, we detected the precipitates using X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) spectrometer. We then identified the carbon source involved in the formation of carbonate precipitates using stable carbon isotopic tracing. Our results will provide new information on how bacteria in drylands contribute to carbon sequestration from the atmosphere.

## 2. Materials and methods

### 2.1. Study site and sampling

Soil samples were collected from the southwestern edge of the Mu Us desert (37°42′N, 107°13′E; 1509 m above sea level). The region has a temperate continental monsoon climate with 275 mm mean annual precipitation (1954–2013), mainly occurring in August and September. The main soil type is Aripsamment (originally from Aeolian sand, with high inorganic carbon levels). The soil (0–20 cm depth) contained 94.8% sand, 4.5% silt, and 0.7% clay (Fa et al., 2016). The soil organic carbon content at a depth of 0–20 cm was about 0.5–3.1 g/m$^2$ (Sun et al., 2016). The vegetation at the research site is dominated by *Artemisia ordosica*, *Leymus secalinus*, *Salix psammophila*, *Agropyron cristatum*, and *Hedysarum mongolicum*. In the middle of July 2016, three 100 m × 100 m sampling sites with typical vegetation cover (*A. ordosica*-dominated, *L. secalinus*-dominated, and *S. psammophila*-dominated) were selected for the collection of soil samples. At each sampling site, three 20 m × 20 m sampling plots were selected with a distance of 20 m from each other. The plant litter on the soil surface was removed before collecting samples. At each soil sampling plot, a total of 12 soil cores was randomly obtained from a depth range of 0–20 cm using a sterilized soil auger (2.5 cm in diameter) and was mixed to create one composite sample. The composite samples were preserved at 4 °C in the refrigerator for no > 2 h before the isolation of bacterial strains.

### 2.2. Isolation and identification of bacteria

For each sample, 1 g of soil was mixed with sterile water (1:10 w/v) by shaking it for 10 min, to distribute the soil bacteria uniformly in the suspension. Afterwards, 1 mL of soil suspension was diluted in 9 mL of autoclaved distilled water, and this suspension was further diluted several times. Based on the preliminary analyses, the appropriate dilution ratio for bacterial isolation was $10^{-3}$. One hundred microliters of this suspension, as the inoculum, was spread on each mineral salt medium (MSM) plate at pH 7.6, and the same amount of autoclaved distilled water without soil suspension was used as the control to remove any potential existing microbes from the distilled water. All plates were incubated at 25 °C for 7 days. The temperature setting, 25 °C, was based on the average soil temperature at a depth of 0–20 cm during sample collection, and this incubation temperature was also used in the previous studies (Delgado et al., 2008; Silva-Castro et al., 2015). MSM contained $Na_2HPO_4{\cdot}2\ H_2O$, 7.8 g/L; $KH_2PO_4$, 6.8 g/L; $Na_2S{\cdot}9\ H_2O$, 0.187 g/L; $MgSO_4$, 0.2 g/L; $Ca(NO_3)_2{\cdot}4\ H_2O$, 0.05 g/L; $NH_4NO_3$, 0.085 g/L; $FeSO_4{\cdot}7\ H_2O$, 0.4 g/L; and 1 mL/L solution of trace elements with 5 g/L $NaHCO_3$ on 1.5% agar (Srivastava et al., 2015). This MSM contained low levels of nutrients, which was used to simulate the relatively low concentrations of nutrients in desert soil (Srivastava et al., 2015). Microbial colonies that appeared on the MSM plates were differentiated morphologically by shape, size, color, smoothness, and other characteristics, and were selected and restreaked onto Luria-Bertani (LB) plates. This process was repeated several times to obtain pure bacterial isolates. Overall, the bacterial isolation experiment lasted for one month. Finally, the colonies were obtained and used for further genetic sequencing analyses.

Genomic DNA (1 μL) samples from the microbial colonies were isolated using the Genome DNA Kit (Qiagen Inc., USA), according to the manufacturer's instructions. 16S rRNA was amplified using PCR analyses with the primer pair 27F/1492R (Andrei et al., 2017). The reaction conditions for amplification were carried out as follows: 5 min initial denaturation at 95 °C, followed by 30 cycles of: 20 s denaturation at 95 °C, 30 s primers annealing at 50 °C, and 3 min extension at 60 °C. The amplified DNA was purified using Qiaquick PCR Purification Kit (Qiagen Inc., USA) and sequenced. The obtained data were compared and analyzed using the data obtained from the GenBank, National Center for Biotechnology Information (NCBI). Phylogenetic analysis of the 16S rRNA genes was inferred by MEGA, version 7.0, with the maximum likelihood method, and bootstrap values were based on the percentages of 1000 replicates (Kumar et al., 2016). Nucleotide sequences have been submitted to the GenBank with the accession numbers MF 136453–136458.

### 2.3. Carbonate precipitation by bacteria

To test the capacity of bacteria to precipitate carbonate, bacterial strains were grown aerobically in 150 mL of liquid growth medium (pH 7.60) in 250-mL lightproof culture flasks in triplicates at 25 °C. Liquid growth medium contained yeast extract (5 g/L), peptone (10 g/L), sodium chloride (5 g/L), and Ca chloride (2 g/L). Before using this liquid growth medium as the culture media, the isolates were incubated in MSM containing Ca chloride (2 g/L). However, no carbonate precipitates were observed, which may be due to the deficiency of required materials for the specific metabolic processes that precipitate carbonate (Hammes and Verstraete, 2002). Details of this preliminary trail were shown in the supplementary materials (Supplementary descriptions of preliminary trail and Fig. A1). To better determine the capacity of bacteria to precipitate carbonate, we used the liquid growth medium that is commonly applied in studies of carbonate precipitation by bacteria owing to the rich substrates for the specific metabolic activity (Banks et al., 2010; Otlewska and Gutarowska, 2016). To eliminate the potential effects of the medium itself, and organics formed by bacterial strains during microbial carbonate formation, control experiments were conducted at the same time by using no bacterial strain inoculation medium (designated as control 1) and autoclaved bacterial strain inoculation medium (designated as control 2) in triplicate for each bacterial strain. To obtain the autoclaved bacterial strains, the bacterial strain samples were sterilized using an autoclave (YXQ-LS-50SII, Boxun, China) at 121 °C for 20 min (Stocks-Fischer et al., 1999). However, determining the effects of autoclaving on the cell surface structure and properties of bacteria is challenging. Usually, the medium inoculated with autoclaved bacteria is used (Cacchio et al., 2003; Rivadeneyra et al., 2004; Silva-Castro et al., 2015), and could be used as one type of control. The number of days required for the formation of crystal carbonates by the bacterial strains was determined based on our preliminary trials (Table A1). Considering the effects of the particular