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CO_2 sequestration by ureolytic microbial consortia through microbially-induced calcite precipitation

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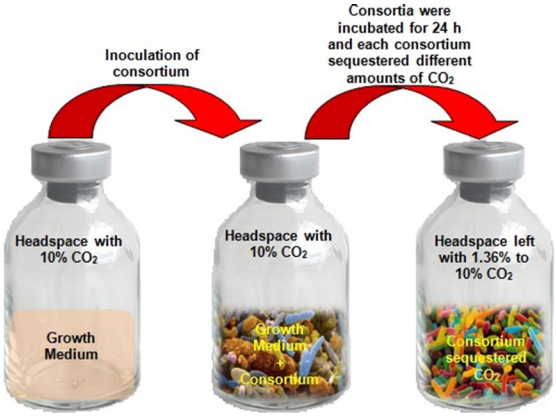
HIGHLIGHTS

- CO_2 sequestration through MICP was investigated.
- Growth conditions were important for selection of ureolytic microorganisms.
- CO_2 sequestration depended on the consortium composition.
- Five genera seem to be involved in CO_2 sequestration.
- Caves and travertines have the potential to be natural carbon sinks.

GRAPHICAL ABSTRACT



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ABSTRACT

Urea is an abundant nitrogen-containing compound found in urine of mammals and widely used in fertilizers. This compound is part of the nitrogen biogeochemical cycle and is easily biodegraded by ureolytic microorganisms that have the urease enzyme. Previous studies, with ureolytic isolates, have shown that some ureolytic microorganisms are able to sequester CO_2 through a process called microbially-induced calcium carbonate precipitation. The present study investigates 15 ureolytic consortia obtained from the "Pamukkale travertines" and the "Cave Without A Name" using different growth media to identify the possible bacterial genera responsible for CO_2 sequestration through the microbially-induced calcite precipitation (MICP). The community structure and diversity were determined by deep-sequencing. The results showed that all consortia presented varying CO_2 sequestration capabilities and MICP rates. The CO_2 sequestration varied between 0 and 86.4%, and it depended largely on the community structure, as well as on pH. Consortia with predominance of *Comamonas*, *Plesiomonas* and *Oxalobacter* presented reduced CO_2 sequestration. On the other hand, consortia dominated by *Sporosarcina*, *Sphingobacterium*, *Stenotrophomonas*, *Acinetobacter*, and *Elizabethkingia* showed higher rates of CO_2 uptake in the serum bottle headspace.

1. Introduction

The increasing concern of global climate change due to increasing emissions of CO_2 has attracted scientists' attention for alternative

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mechanisms to sequester CO_2. One way for decreasing the atmospheric CO_2 concentrations is converting the CO_2 into carbonate minerals, because mineral carbonation has been presented as a geologically stable and environmentally safe way to store carbon (Ramanan et al., 2009). The major issue with spontaneous chemical carbonate mineral formation is that this process tends to have slow reaction rates and is highly dependent on pH (Zhu and Logan, 2014). Biological mineral carbonation formations, called microbially-induced carbonate precipitation (MICP), on the other hand, have recently been suggested to participate in CO_2 sequestration (Okwadha and Li, 2010). This biological process can frequently involve urea, which is commonly found in fertilizers and in the urine of mammals.

Microorganisms involved in the degradation of urea are called ureolytic microorganisms. During the degradation of urea, which is an organic compound, CO_2 and ammonia are produced. Because the ammonia leads to an increase in pH, the CO_2 produced during urea hydrolysis is converted to CO_3^{2-}. In environments where unsaturated calcium concentrations and microorganisms involved in MICP are present, the microorganisms will serve as a catalyst of the reaction of the carbonate with calcium ions to form calcium carbonate precipitates. The chemical reactions for ureolysis, CO_2 dissolution at different pH values and MICP can be seen in our previous study (Okyay and Rodrigues, 2015).

The best model environmental systems to investigate the MICP are karstic environments, such as caves and travertines, since these environments have abundance of calcium ions (Kumaresan et al., 2014) and are frequently contaminated by urea through different sources, such as through bats' and other mammals' urine (Johnston et al., 2012), and from seasonal or continuous water infiltration from the surface (Baker and Fairchild, 2012; Jameson and Alexander, 1994; Ortiz et al., 2013). Besides the urea and calcium, the presence of CO_2 has also been shown to play an important role in the MICP process (Okyay and Rodrigues, 2015).

The MICP process has two possible CO_2 sources for the calcite precipitation to happen: i) CO_2 dissolution present in the air or from dissolved minerals and ii) CO_2 produced during ureolysis and respiration. In the latter, the main question is whether the amount of CO_2 produced by bacterial metabolism will exceed the bacterial capability to sequester CO_2 through MICP, which would lead to no significant CO_2 sequestration.

A recent study demonstrated that some environmental microbial isolates are able to sequester not only the CO_2 produced by their own microbial metabolism, but also a 10% (v/v) excess of CO_2 present in the headspace of serum bottles (Okyay and Rodrigues, 2015). In this study, it was also demonstrated that the amounts of calcite precipitated and CO_2 sequestered through MICP was directly dependent on the microbial species. From this study, we hypothesized that the dominance and abundance of microbial populations involved in MICP will determine whether a consortium can successfully sequester CO_2. The rationale for this hypothesis is that in a consortium some species will not be able to perform MICP or will have poor MICP activity to sequester enough CO_2, since different species have different metabolic activities. For instance, if populations incapable of MICP are dominant and more abundant in a specific consortium, they will produce excessive amounts of CO_2 through respiration, which will yield a low or no significant CO_2 sequestration by a less abundant microbial population involved in MICP in this same consortium.

In order to investigate this hypothesis, 15 ureolytic consortia were obtained from the 'Cave Without A Name' and 'Pamukkale travertines' using 13 different growth media for ureolytic microorganisms. The amounts of CO_2 sequestered through MICP by the consortia were determined via CO_2 mass balance using the amounts of calcite precipitated, CO_2 gas left in the headspace of the serum bottles and by taking into consideration the CO_2 chemical speciation in the growth media. The amount of CO_2 sequestered by each consortium was further correlated to the microbial composition and abundance to identify groups of microorganisms playing a potential role in the MICP and CO_2 sequestration. The microbial composition and abundance was determined using deep sequencing (Illumina MiSeq). This study is the first one to: i) evaluate the potential CO_2 sequestration of ureolytic consortia involved in MICP, ii) distinguish the biotic and abiotic factors affecting CO_2 sequestration during MICP, and iii) employ high-throughput deep-sequencing to characterize ureolytic microbial consortia involved in MICP. Lastly, this investigation identifies genera that could be potentially involved in CO_2 sequestration during MICP in karstic environments.

2. Materials and methods

2.1. Sample collection and enrichment of consortia

Water samples were collected aseptically in sterile 0.5 L polyethylene containers from two karstic locations; 'Pamukkale travertines' (Denizli, Turkey) (37°54′59″ N, 29°07′02″ E) and 'Cave Without A Name' (Boerne, TX, USA) (29°47′40″ N, 98°43′55″ W) in December 2011 and March 2012, respectively. They were transported at 4 °C and kept at 4 °C until processed. The water samples were characterized according to the standard methods of water analysis (APHA, 2005).

Calcite precipitating consortia were enriched in 13 different growth media (10%, v/v). The medium compositions used in the enrichment process of this study are described in Table S1.

All experiments, including the initial consortium enrichments, were incubated aerobically at 150 rpm (INNOVA 44) for 3 d at 28 °C and 20 °C for travertine and cave samples, respectively. These temperatures were based on the local temperatures during sampling. TR and CV abbreviations correspond to travertine and cave consortia, respectively.

2.2. Determination of urease activity

The urease activity of each consortium was determined as previously described (Okyay and Rodrigues, 2013). Briefly, the optical density (OD) of each consortium at 560 nm was measured every 30 min for 24 h with a 96-well plate reader (Biotek). The data obtained was used to calculate the enzymatic activity (U mL^{-1}) for each consortium with a standard curve obtained from the purified Jack Bean urease enzyme.

2.3. Investigation of calcium carbonate precipitates

In this study, two investigations were done: i) weigh directly the calcium carbonate precipitates after 96 h incubation; and ii) analyze the calcium carbonate precipitates by a scanning electron microscopy (SEM) and an energy dispersive X-ray spectroscopy (EDS) (JEOL JSM-6010LA).

In the first investigation, the amount of calcite precipitated by each consortium at the end of the incubation period was determined. For each consortium, culture bottles having growth medium containing calcium source were incubated at 150 rpm for 96 h. Next, the solutions were centrifuged in 50-mL centrifuge tubes at 12,857 *g* for 10 min, and the supernatants were discarded. The pellets were freeze-dried, and then the dry pellets were weighed, which corresponded to 'microbial biomass + calcite precipitate'. In order to obtain the amounts of calcite precipitated, we performed, in parallel, identical sets of experiments without calcium to determine the weight of the 'microbial biomass' only. This amount of microbial biomass was subtracted from the total (microbial biomass + calcite precipitates). Controls were set up with growth medium containing calcium source, but no cells, to ensure that no spontaneous chemical precipitation was happening in the growth media during incubation. All experiments were performed in triplicate. The mean values of the amounts of calcite precipitated and their respective standard deviations were calculated for each consortium. Finally, the calcium carbonate precipitates were analyzed by SEM and EDS using a method previously described (Okyay and Rodrigues, 2014).

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