Subcloning of urease enzyme in *Sporosarcina ureae*

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BACKGROUND

Earthquakes devastate many lives each year, causing death, collapsing infrastructure, and forcing relocation. One of the factors leading to significant damage is liquefaction, a process in which the ground adopts a quick-sand like texture due to decreased strength and stiffness as a result of earthquake shaking or other rapid loading.

Microbially induced calcite precipitation (MICP) is an emerging technique that could increase soil resistivity in earthquake and landslide prone zones[1,2]. MICP takes advantage of the by products of bacterial metabolic pathways to form calcite precipitation throughout soil matrix, increasing soil strength and stiffness[3]. Thus, genetically optimizing biogenesis of $CaCO_3$ in ureolytic bacteria, like *Sporosarcina ureae*, could result in a practical and earth-friendly solution for improving ground stability, mitigating the effects of liquefaction.

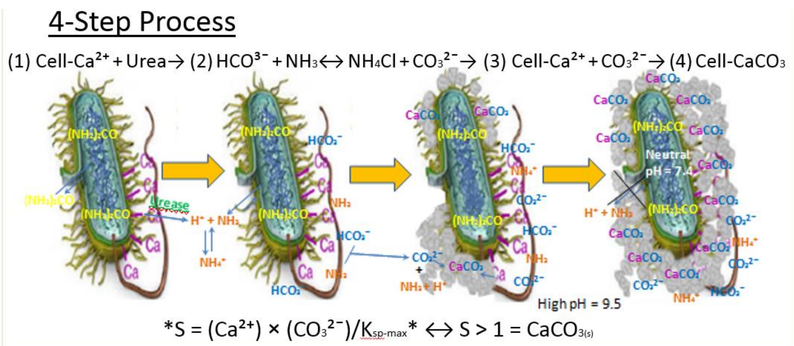


Figure 1: Proposed mechanism of $CaCO_3$ formation based on the urease enzyme activity in *S. ureae*

OBJECTIVES

Ultimately, this project aims to contribute to Masters Candidate J. Whitaker's ongoing effort to (1) finalize a recombinant UreA-G strain for potentially improving ureolytic capability for biocement production, (2) analyze the roles of coenzymes and hoenzymes expression for ureolysis. and (3) confirm successful overexpression of urease in first *E. coli* and *S. ureae*. to improve biogenesis of $CaCO_3$ via urea degradation.

MATERIALS & METHODS

UreA-G operon, which contains genes coding for urease enzyme and other necessary coenzymes, was subcloned into several bacteria strains in order to compare different levels of urease protein expression. Several plasmid vectors were used to fully subclone the operon and to ensure the stability & the expression of urease.

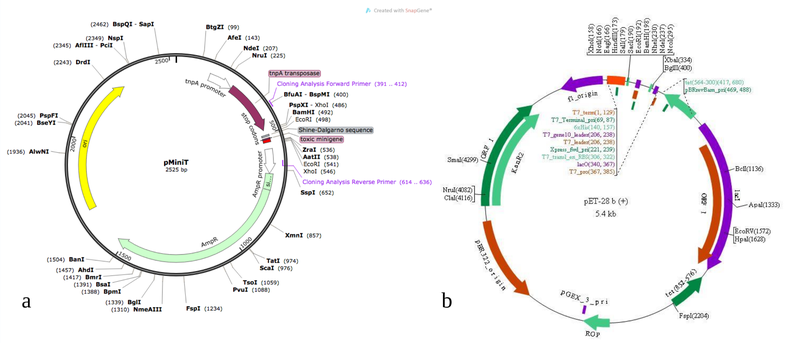


Figure 2: (left to right) a- plasmid pMiniT has a toxic mini-gene that was utilized to ensure two parts of the operon (UreA-C and UreD-G) were both subcloned in to *E. coli.;* b- plasmid pET28b has viral T7 promoter region which is only recognized by certain strains and is IPDG induced

	Strain	Plasmid	Operon	Rationale
1	*E. coli dH5α, dH5β*	pMiniT	UreA-C, UreD-G	• Testing the effects of different coenzyme • High efficiency cloning method, especially for large operon (UreA-G ≈5kbp)
2	*E. coli Top10*	pET28b	UreA-C, UreD-G	• Stabilize vector in non-expressing bacteria before testing for protein output
3	*E. coli BL21(DE3)*	pET28b	UreA-G	• Combine operon into one vector • Stabilize and express (vector IPTG induced)
4	*E. coli Top10*	pLAM1	UreA-G	• Stabilize vector in *E. coli*
5	*E. coli BL21(DE3)* *Bacillus subtilis* *S. ureae*	pLAM1	UreA-G	• Comparison of operon expression and urease level in different strains • *S. ureae* recombinant predicted to have highest level of urease because it naturally has highest level of ureolytic activity

RESULTS

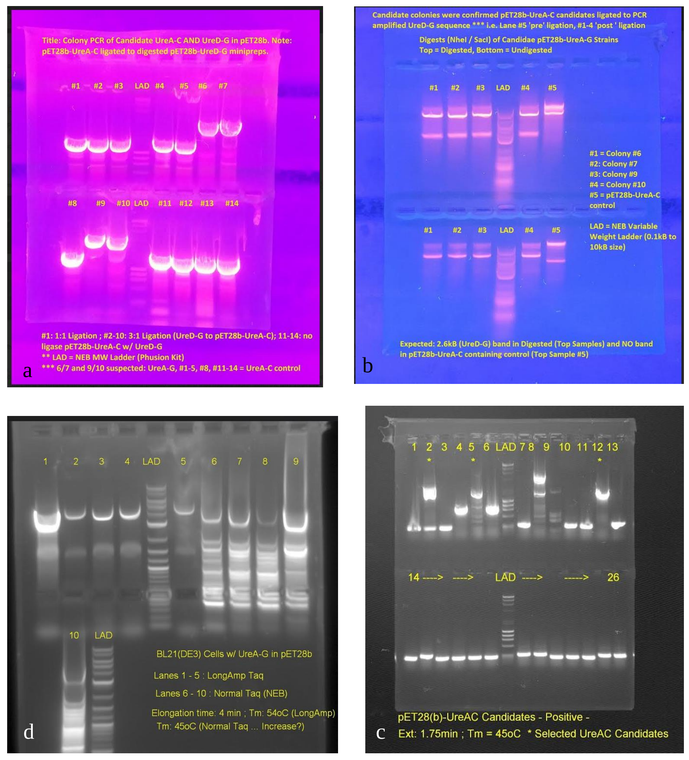
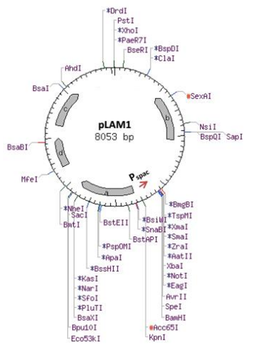


Figure 3: Electrophoresis gel of colony PCR at different stages of subcloning (clock wise from top left) ligating Ure D-G to pET28b Ure A-C (refer to chart- steps *2 to 3*) in **a** & **b**, attempting different ratio of UreD-G to to pET28b Ure A-C in **a**, confirming that selected colonies have ligated UreA-G in *E. coli BL21(DE3*) in **b**; confirming the stability of UreA-C in pET28b (refer to chart- step *2*) in **c**; comparing different polymerase (Taq vs Taq long-amp) in amplifying pET28b in *E. coli BL21(DE3)* in preparation for transformation of UreA-G to pLAM1 (refer to chart- steps *3 to 4*)

CONCLUSION



Operon UreA-G has been successfully subcloned into pET28b plasmid vector and is stable in *E. coli BL21(DE3)*. This strain is ready to express the urease enzyme when IPDG inducer is introduced (step 3 completed).

To finalize a recombinant strains, UreA-G needs to be subcloned into pLAM1 and transformed into *B. subtilis*, *S. ureae*, and *E. coli BL21(DE3).* Once stable, IPDG can be used to induce urease enzyme production to compare the efficiency of different strains in biogenesis of $CaCO_3$ precipitate.

Figure 4: pLAM1 vector has a replication of origin that both Gram + and – bacteria recognize

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Figure 1: J.Whitaker "Bio-Mediated $CaCO_3$ Production as a Method for Improving Liquefaction Resistance of Sandy Soils." *University of Ottawa UROP Poster* (2012).
Figure 2a:http://www.snapgene.com/resources/plasmid_files/basic_cloning_vectors/pMiniT/ (Web) Mar 09, 2016.
Figure 2b:: http://www.biovisualtech.com/bvplasmid/pET-28_b_(+).htm (Web) Mar 09, 2016.

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