

## REQUIMTE/CQFB - Chemistry Department

### Arsenic Detoxification Mechanism in *D. alaskensis* G20

Bioin – BioProt – Bacterial Mechanisms of Environmental Adaptation Group  
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2010 – PhD Student in Sustainable Chemistry  
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## Objectives

Recently it was reported that *D. desulfuricans* G20 possesses an arsenic resistance operon with the following arrangement *arsRBC2C3*. This operon contains two different arsenate reductases (ArsC2 and ArsC3), that putatively convert intracellular As(V) to As(III), a membrane protein responsible for As(III) extrusion (ArsB) and an As(III) inducible transcription regulator (ArsR). We are interested on the biochemical and functional characterization of the two arsenate reductases present in the *arsRBC2C3* operon. We expect to determine the co-factor requirement (thioredoxin or glutaredoxin) as well as the kinetic parameters of these enzymes.

## Methodology

- Cloning, overexpression and purification of the two ArsCs present in the *arsRBC2C3* operon and their cofactor requirements.
- Determination of molecular mass, molar extinction coefficient, isoelectric point.
- Kinetic studies.
- Complementation arsenic resistance assays in *E. coli*.
- Transcriptomic studies.
- Backbone resonance assignment of *arsC* – NMR.

## Expected Results

- Get insights on the biological relevance of both arsenate reductases in *D. alaskensis* G20 as well as the mechanism of As(V) reduction in sulfate reducing bacteria (SRB).
- The complementation assays in *E. coli* will elucidated the importance of each arsenate reductase on the arsenic resistance mechanisms in SRB.
- ArsC will be labeled with  $^{13}\text{C}$  and  $^{15}\text{N}$  in order to perform NMR experiments for backbone resonance assignment.
- Transcriptomic studies will enable the identification of genes whose expression is affected by the presence of arsenate in the growth culture medium



Fig. 1 – Arsenic Resistance operon in *D. alaskensis* G20

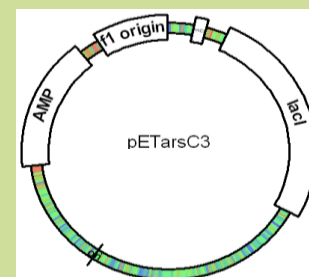


Fig. 2 – Expression vector of *arsC*

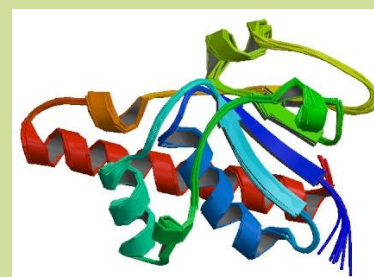


Fig. 3 – Structure of *arsC* from *B. subtilis* 1Z2D.PDB