Arsenite Oxidase Genes From *Alcaligenes faecalis* to *E.coli* : Gateway Cloning

Authors: Ashley Compton, Mitchell Moseng, Kirk Mecklenburg, Dr. Gretchen Anderson

Indiana University South Bend Department of Chemistry and Biochemistry, South Bend, Indiana, USA 46634

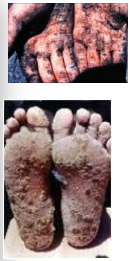
Abstract

Groundwater contamination by arsenic has become a global environmental catastrophe. Arsenic is a naturally occurring toxic element that is found in most water and soil. High concentrations of arsenic have been connected to disease such as cancers, skin lesions (Figure 1), hypertension, and chromosomal abnormalities (Dastgiri et *al.* 2010, Smith et *al.* 2000). Arsenicosis is the broad range of diseases caused by arsenic poisoning. Among the 125 million inhabitants within Bangladesh, up to 77 million are at risk of suffering from arsenicosis due to contaminated drinking water (Smith et *al.* 2000).

One of the few bacteria to develop a resistance to arsenic is the bacterium *Alcaligenes faecalis*. One known resistance mechanism is the bacterial arsenite oxidase enzyme (Cai 2009, Figure 2) which detoxifies arsenic by converting arsenite into arsenate. Arsenate is 200x less toxic but also carries a negative charge. This charge is advantageous because it allows arsenate to be removed from water.

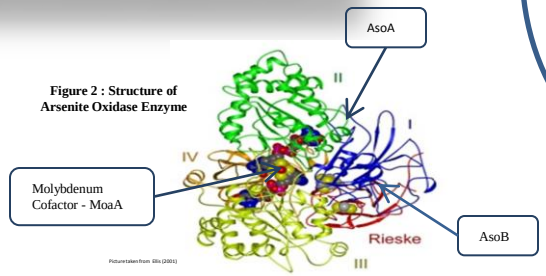
The purpose of this project was to clone the genes necessary for the over-expression and biochemical characterization of the arsenite oxidase enzyme, whose features may be used in decontamination of water around the world.

Figure 1 : Effects of arsenicosis



The arsenite oxidase enzyme is a heterodimer (Figure 2). Three genes, AsoA, AsoB, and MoaA, are needed to supply instructions for making the enzyme and inserting the molybdenum cofactor.

Figure 2 : Structure of Arsenite Oxidase Enzyme



Experimental Procedure

PCR – Selectively amplify arsenite oxidase genes based on designed primers

Gel electrophoresis – Size comparison of experimentally amplified DNA segments to the known size of genes in GenBank (Figure 3)

Recombination with genes of interest and gateway pENTR vector by Gateway protocol.

Determination of proper orientation in entry vector – Vectors were tested to ensure the genes were correctly oriented by a restriction enzyme digest with Eco RI (figure 4)

DNA sequencing and sequence analysis (figure 5)

Gateway Cloning

Gateway cloning is an extremely efficient cloning method (upper 90% range). The increased efficiency is due to the lethal ccdB gene, antibiotic resistance, and the ease of expression into vector systems due to the unique recombination sites. This allows highly effective selection of productive clones.

Step 1 : BP reaction — Primers are designed with attB recombination sites to flank the DNA of interest. The attB sites are recombined with the attP sites on the pENTR vector to produce the entry clone which also contains attL recombination sites.

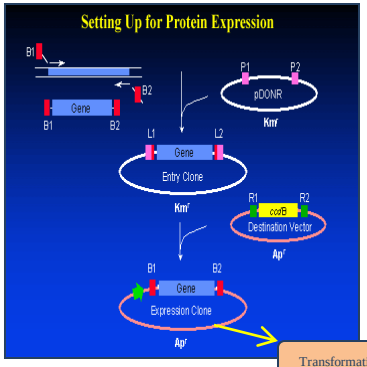
* Entry clone can be used to make more entry clones or to continue to LR reaction and clone the DNA of interest

Step 2 : LR reaction — The attL sites on the entry clone are recombined with the attR sites on the destination vector.

Step 3 : Resolution of Expression Vector — The destination vector is resolved into two molecules, the expression vector containing the DNA of interest and a by-product molecule containing the ccdB gene.

* The ccdB gene will not amplify and prevents growth of daughter cells that did not inherit the vector .

Step 4 : Transformation into E.coli



Transformation

Proper genes were amplified and cloned into entry vector in the proper orientation

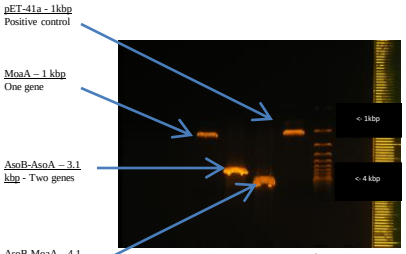


Figure 3: Gel electrophoresis confirms the isolation of proper genes by size. [Gene fragments from left to right: MoaA (one gene), AsoB-AsoA (two genes), AsoB-MoaA (three genes), Positive control (pET-41a)]

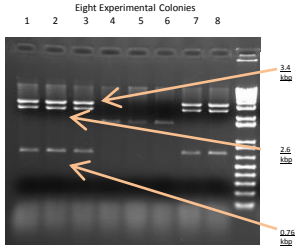


Figure 4: Eco RI restriction digest of AsoB-MoaA (three genes) confirms genes were cloned into proper orientation

Sequencing:

The cloned genes were sequenced to determine if there were any mismatches (mistakes) during cloning. Forward and reverse sequences had 100% similarity . However, alignment of the *A. faecalis* DNA from GenBank and our experimental sequence returned a 99% match. This alignment showed three possibly significant mismatches (figure 5). Although there were base mismatches at #1 and # 2, the same amino acids were coded, respectively, leucine and glycine. This resulted in no change to the protein. At site #3, the base mismatch would result in a serine within the cloned enzyme rather than the leucine in the native enzyme. Since serine is polar and leucine is not this is a significant change that can affect the three-dimensional shape of the protein.

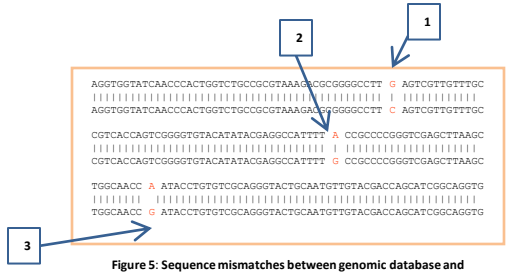


Figure 5: Sequence mismatches between genomic database and experiment sequence.

Conclusion

- All three genes, AsoA, AsoB, and MoaA, were successfully amplified from *A. faecalis*
- The three gene segment (AsoB-MoaA) was cloned into the Gateway pENTR vector and confirmed to be in complete form and in correct orientation
- Sequencing indicated three base mismatches but only one amino acid mismatch
- Future research will be to mutate the mismatch back to native form and express the protein

Acknowledgements:

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References:

Dr. Gretchen Anderson, Indiana University South Bend, Department of Chemistry and Biochemistry

Cai, Lin , Liu, Guanghui, Rensing, Christopher, Wang, Gejiao (2009). Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiology, 9(4).

Dastgiri, S., Mosaferi, M., Fizi, M. H., Olfati, N., Zolali, S., Pouladi, N., & Azarfam, P. (2010). Arsenic Exposure, Dermatological Lesions, Hypertension, and Chromosomal Abnormalities among People in a Rural Community of Northwest Iran. *Journal of Health, Population, & Nutrition*, 28(1), 14-22.

Ellis, Paul J., Conrads, Thomas, Hille, Russ, Kuhn, Peter (2001). Crystal Structure of the 100kDa Arsenite Oxidase from *Alcaligenes faecalis* in Two Crystal Forms at 1.64 Å and 2.03 Å. *Structure*, Vol 9, 125-132

Smith Allan H., Lingas Elena O., Rahman Mahfuzar (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ.*, 78(9): 1093-1103.