Final Report
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Biosynthetic Approaches to Isotope Enrichment for Applications in Neutron Scattering and High Field NMR Spectroscopy: Methylotrophic Expression Systems

Limitations in current isotopic labeling methods present a substantial bottleneck for the application of advanced structural techniques to many important biochemical problems. New tools are required to efficiently produce the necessary labeling patterns in biochemical precursors and incorporate them into protein molecules for structural studies. This project proposed involved one aspect of this problem, the development of expression vectors for a methylotrophic bacterium, *Methylobacterium extorquens* AM1. If high-level, efficient expression could be obtained in such a bacterium, it would be possible to use low-cost ^{2}H- and/or ^{13}C-labeled substrates such as methanol to label proteins. The Lidstrom laboratory at the University of Washington worked closely with our collaborators at Los Alamos National Laboratories (Drs. Cliff Unkefer and Jill Trewhella) in the development and use of these vectors.

1. Overexpression of a target gene, bacterial dehalogenase. This enzyme was expressed in *Methylobacterium extorquens* AM1 using a high level methanol-inducible promoter, the *mxaF* promoter. High expression was achieved, but most was in an insoluble form. We expressed this protein in a mutant lacking polybetahydroxybutyrate granules, and high expression was achieved, up to 10% of the total soluble protein. The recombinant protein was purified and shown to be active, with characteristics similar to the enzyme produced in *E. coli*. This work was published (FitzGerald et al., 2003).

2. Development of regulated expression systems
A number of regulated promoters were tested in *M. extorquens* AM1, the most promising of which appeared to be the *E. coli lac* promoter coupled to the LacIq regulator. The repressor was shown to be active and a chromosomal insertion construct was generated that repressed the low-level *lac* promoter activity in *M. extorquens* AM1. However, IPTG induced this system only poorly. A number of studies were carried out leading to the conclusion that IPTG entered the cell but was exported by one or more export pumps. Target genes for such pumps were mutated but none of these showed increased induction. A number of methods were used to permeabilize the cell, and a 2-fold increase in induction was obtained with one of these.

The activity of the *lac* promoter was increased by inserting a recently-identified *M. extorquens* AM1 enhancer element upstream. The promoter increased in activity 5-6 fold with this addition.

In summary, we have developed a suite of expression tools and host mutant strains for expressing a variety of heterologous proteins in this methylotroph. These are now available for testing by our LANL collaborators in labeling reactors to obtain labeled proteins of interest. We have published these results along with other literature in a

review chapter highlighting protein expression in methylotrophs (FitzGerald & Lidstrom, 2004).

Publications

Fitzgerald KA, Lidstrom ME. 2003. Overexpression of a heterologous protein, haloalkane dehalogenase, in a poly-beta-hydroxybutyrate-deficient strain of the facultative methylotroph *Methylobacterium extorquens* AM1. Biotechnol Bioeng. 81(3):263-8.

K. Fitzgerald and Lidstrom, M.E. 2004. Heterologous protein expression in methylotrophic bacteria. In, Expression Technologies: Current Status and Future Trends, F. Baneyx, ed., Horizon Scientific Press.