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David B. Wilson

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The students supported by this training grant all worked on a project to develop an E. coli host-plasmid, reactor combination that would give continuous production of any protein whose structural gene was cloned into the plasmid. The first student supported by this grant studied the excretion of Beta-lactamase by E. coli transformed by either plasmid pKK or pKN both of which contain a tac promoter inserted between the Eco RI and Hind III sites of pBR322 oriented so that transcription initiated at the tac promoter reads through the Beta-lactamase structural gene. The second student supported by this grant worked on a project to obtain secretion of the cytoplasmic protein, E. coli galactokinase by constructing fusions between the signal sequence and various amounts of the N-terminal sequence of mature Beta-lactamase and galactokinase. The last student Lisa Laffend, worked on a project to obtain secretion of the cytoplasmic protein E. coli xylose isomerase, by constructing a set of hybrid proteins which contained a modified ompA signal sequence jointed to (continued on reverse side)

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the N-terminus of xylose isomerase under the control of the lac promoter of plasmid pUC18.

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Training in Biotechnology at Cornell University
Final Report

David B. Wilson

January 1990

U.S. Army Research Office

DAAL03-86-6-0030

Cornell University

The students supported by this training grant all worked on a project to develop an *E. coli* host-plasmid, reactor combination that would give continuous production of any protein whose structural gene was cloned into the plasmid. The first student supported by this grant, George Georgiou, studied the excretion of β-lactamase by *E. coli* transformed by either plasmid pKK or pKN both of which contain a *tac* promoter inserted between the *Eco* RI and *Hind* III sites of pBR322 oriented so that transcription initiated at the *tac* promoter reads through the β-lactamase structural gene. The second student supported by this grant, Susan Egan, worked on a project to obtain secretion of the cytoplasmic protein, *E. coli* galactokinase by constructing fusions between the signal sequence and various amounts of the N-terminal sequence of mature β-lactamase and galactokinase. The last student supported by this grant, Lisa Laffend, worked on a project to obtain secretion of the cytoplasmic protein *E. coli* xylose isomerase, by constructing a set of hybrid proteins which contained a modified ompA signal sequence joined to the N-terminus of xylose isomerase under the control of the *lac* promoter of plasmid pUC18. The plasmids constructed were introduced into *E. coli* strain KL16-23 which is unable to grow on fructose because of mutations in the genes for fructose enzyme II and mannose enzyme II. The transformants were plated on minimal fructose plates containing IPTG to induce xylose isomerase synthesis and select for any cells containing plasmids that secreted xylose

isomerase which would catalyzed the isomerization of fructose to glucose and thus allow growth.

The work of George Georgiou while he was supported by the grant has been reported in three publications as well as his Ph.D. thesis. George is an assistant professor of chemical engineering at the University of Texas in Austin and was awarded a presidential young investigator award.

Susan Egan succeeded in constructing fusions between β-lactamase and *E. coli* galactokinase and these fusions retain galactokinase activity, however none of them appeared to be secreted by. *E. coli*. This in in contrast to the situation in *Streptomyces lividans* where it was shown that an *S. lividans* signal sequence allowed complete secretion of *E. coli* galactokinase. Unfortunately the workers did not test their construction in *E. coli*, so it is not known if the differences between our work and theirs are due to the different signal sequences used or differences in the organism's secretion machinery. Susan decided to change projects and is now completing her graduate training in Dr. Valley Stewart's laboratory in the department of microbiology.

She was replaced on the grant by Lisa Laffend, a graduate student in chemical engineering. Lisa has finished the plasmid construction started by Susan in which an *E. coli* xylose isomerase gene with a mutation in its N terminal coding region to create an *Nhe*I site was cloned into pUC18. Then a 64 base pair synthetic DNA which would code for the omp A signal sequence but which had all 4 bases present in several positions just before the leader peptidase cleavage site was inserted into the *Hind* III - *Nhe* I sites of the pUC18 xyl A plasmid to construct a set of hybrid genes coding for xylose isomerase-signal sequence fusion proteins. This set of plasmids was introduced into *E. coli* strain K16-23 and transformants were selected on LB amp plates. Amp positive transformants were then

tested for growth on fructose minimal plates containing different levels of IPTG. Two strains were identified that appeared to grow on fructose and plasmid DNA prepared from each stain gave fructose positive transformants when strain KL16-23 was retransformed with the plasmids.

Publications:

1. Georgiou, G., Chalmers, J.J. and Schuler, M.L. Continuous Immobilized Recombinant Protein Production from *E. coli* Capable of Selective Protein Excretion: A Feasibility Study. Biotech. Progress, Vol 1(1), March 1985.

2. Georgiou, G., Telford, J.N., Shuler, M.L. and Wilson, D.B. Localization of Inclusion Bodies in *Escherichia coli* overproducing β-lactamase or Alkaline Phosphatase. Appl. Environ. Microbiol. 52, 1157-1161 (1986)

3. Georgiou, G., Shuler, M.L. and Wilson, D.B. Release of Periplasmic Enzymes and Other Physiological Effects of B-Lactamase Overproduction in *Escherichia coli*. Biotech. and Bioeng. 32, 741-748 (1988).

4. Georgiou, G., Palumbo, T., Wilson, D.B. and Shuler, M.L. Effect of Alkaline Medium on the Production and Fxcretion of β-Lactamase by *Escherichia coli*. Biotech. Letters 10(6), 377-382 (1988).

5. Georgiou, G. Thesis "Inducible Overproduction and excretion of a periplasmic protein (β-Lactamase) in *Escherichia coli*.." (1987)

Personnel Supported:

Dr. George Georgiou, Ph.D. 1987
Susan Egan
Lisa Laffend