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PROGRESS REPORT
"Physiology and Genetics of Metabolic Flux Control in *Zymomonas mobilis*"
Grant No. DE-FG02-90ER20002

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Tyrrell Conway

Tyrrell Conway, Principal Investigator

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X. PROGRESS REPORT

A. Specific Objectives of Current Grant

The research funded by the Department of Energy, beginning June 1, 1990, deals with the physiology and genetics of glycolytic flux control in *Zymomonas mobilis*. Two fundamental biological questions are being addressed: First, how do the enzymes of glycolytic pathways act in concert to regulate metabolic flux? Second, what is the role of gene expression in regulating high level synthesis of the glycolytic enzymes in a balance that allows proper glycolytic flux control? The specific objectives of the grant are as follows: **1.** To clone the structural and regulatory regions of the *Z. mobilis* genes encoding glucose-6-phosphate dehydrogenase, phosphoglucose isomerase, enolase, 6-phosphogluconate dehydratase, 2-keto-3-deoxy- 6-phosphogluconate aldolase, glucokinase and fructokinase. **2.** To characterize the structure of these genes with respect to nucleotide sequence, transcriptional initiation sites, promoter location, evolutionary relatedness to similar genes from other organisms, and organization of these genes on the genome. **3.** To investigate the effects of genetically engineered alterations in the levels of the cloned enzymes on metabolic flux and cell growth. **4.** To study transcriptional and post-transcriptional regulation of the genes encoding the enzymes of the Entner-Doudoroff pathway. The first two specific objectives have now been fully completed. Significant progress has been made on the fourth objective and work on the third objective is well underway.

B. Summary of Current Research

The long-term goal of this and related work is to understand the role of gene expression in regulating glycolytic enzyme synthesis in a balance that allows proper glycolytic flux control. Prior work on *Zymomonas mobilis* is extensive. The biochemistry and physiology of glycolysis in *Z. mobilis* is well understood, providing a meaningful background for these studies.

During the past two years the seven genes targeted for study in this laboratory have been cloned and sequenced, and molecular details of regulation have been investigated. It is clear that glycolytic enzyme synthesis is coordinated to prevent the build up of toxic metabolic intermediates. The genetic mechanisms responsible for regulating balanced expression of the Entner-Doudoroff and glycolytic genes in *Z. mobilis* are beginning to be understood. Several layers of genetic control, perhaps in a hierarchal arrangement, act in concert to determine the relative abundance of the glycolytic enzymes. These genetic controls involve differential translational efficiency, highly conserved promoter sequences, transcription factors, differential mRNA stabilities, and nucleolytic mRNA processing.

The serendipitous cloning of the glucose facilitator, *glf*, as a result of linkage to several other genes of interest will have a significant impact on the study of *Z. mobilis* metabolism. In fact facilitated diffusion of glucose in *Z. mobilis* may represent the only such uptake system known to exist in bacteria. The glucose facilitator is being characterized in a genetically reconstituted system in *E. coli*. Molecular genetic studies in *Z. mobilis* indicate that the ratio of *glf* expression to that of *glk*, *zwf*, and *edd* is carefully regulated, and suggests a critical role in metabolic control.

Regulation of glycolytic gene expression in *Z. mobilis* is now sufficiently well understood to allow use of the glycolytic genes as tools to manipulate specified enzyme levels for the purpose of analyzing metabolic flux control. The critical genes have been subcloned for stable expression in *Z. mobilis*. They have been placed under control of a regulated promoter system involving the *tac* promoter, the *lacI* repressor, and gene induction in *Z. mobilis* by IPTG. In order to fully assess the effects of genetically engineered changes in enzyme levels, HPLC methods have been developed that allow quantitation of virtually all of the metabolic intermediates in the cell pool. These experiments are in progress.

C. Specific Objective 1.

The *Zymomonas mobilis* genes that encode glucose-6-phosphate dehydrogenase (*zwf*), 6-phosphogluconate dehydratase (*edd*), and glucokinase (*glk*) were cloned independently by genetic complementation of specific defects in *E. coli* metabolism and found to be closely linked (Barnell, et. al., 1990). In addition, the glucose facilitator, *glf*, was cloned along with these first three genes (Barnell, et al., 1990). The *Z. mobilis* gene encoding phosphoglucose isomerase (*pgi*) was cloned by genetic complementation of an *E. coli pgi* mutant (Hesman, et. al., 1991). The *eda* gene that encodes 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase of the Entner-Doudoroff pathway was cloned from *Z. mobilis* by genetic complementation of an *E. coli* mutant (Conway, et. al., 1991). The *Z. mobilis frk* gene, encoding fructokinase, was cloned by using a novel enzyme activity-based detection plate (Zembrzuski, et. al., 1992). The *Z. mobilis eno* gene that encodes enolase was cloned by genetic complementation of an *E. coli* mutant (Burnett, et al., submitted for publication). The identity of each of these cloned genes was confirmed by genetic complementation, biochemical assay, and by comparison to the known N-terminal amino acid sequences of the purified enzymes (kindly provided by R.K. Scopes).

D. Specific Objective 2.

1. Evolutionary relatedness. Each of the cloned genes have now been completely sequenced. The *Z. mobilis* 6-phosphogluconate dehydratase sequence was the first for this enzyme from any source (Barnell, et al., 1990). Interestingly, the deduced amino acid sequence is similar to that of *E. coli* dihydroxyacid dehydratase. 6-Phosphogluconate dehydratase is highly conserved in *Z. mobilis* and *E. coli* (Egan, et al., 1992). The glucose-6-phosphate dehydrogenase was the first sequenced from a bacterium and resembles other analogous enzymes (Barnell, et al., 1990). The glucokinase is extremely unusual in lacking a consensus glucose binding site and is dissimilar to other hexokinases (Barnell, et al., 1990). Likewise, the fructokinase is dissimilar to other hexokinases (Zembrzuski, et al., 1992). The *Z. mobilis* KDPG aldolase is similar to the *Pseudomonas cepacia* enzyme (Conway, et al., 1991) and the *E. coli* enzyme, which is identical to 2-keto-4-hydroxyglutarate aldolase (Egan, et al., 1992). The phosphoglucose isomerase is similar to other analogous enzymes (Hesman, et al., 1991). Enolase from *Z. mobilis* is also highly conserved (Burnett, et al., submitted for publication). Nucleotide sequence analysis established that the *zwf*, *edd*, and *glk* genes are clustered on the genome with an additional open reading frame in this region that has significant amino acid identity to the *E. coli* xylose-proton symporter and the human glucose transporter. On the basis of the deduced primary amino acid sequence, this gene is believed to encode the *Z. mobilis* glucose facilitated diffusion protein, *glf* (Barnell, et. al., 1990). Genetic reconstitution of glucose transport in appropriate *E. coli* mutants has provided proof that *glf* does indeed encode the transporter (see below).

2. Gene organization. All eight of the characterized genes are present in single copies on the genome. The *glf*, *zwf*, *edd*, and *glk* genes are clustered on the genome, in that order, and form an operon (Barnell, et al., 1992). The *glf* and *zwf* genes are separated by 146 base pairs. The *zwf* and *edd* genes overlap by 8 base pairs and their expression may be translationally coupled. The *edd* and *glk* genes are separated by 203 base pairs. The four genes of the operon are co-transcribed on a 6.14 kb mRNA. Such an arrangement of the genes that govern glucose uptake and the first three steps of the Entner-Doudoroff glycolytic pathway provides the organism with a mechanism for carefully regulating the levels of the enzymes that control carbon flux into the pathway. The *eda*, *pgi*, *frk*, and *eno* genes are all monocistronic. The *eda* gene is not tightly linked to the *edd* gene, as in other bacteria. No evidence for linkage to the other glycolytic genes has been obtained.

3. Transcript mapping and identification of a consensus promoter. A single site of transcriptional initiation for the polycistronic *glf-zwf-edd-glk* message was mapped by primer

extension and nuclease S1 protection analysis (Barnell, et. al., 1992). The *eda* gene is transcribed from a single promoter that was mapped with respect to the transcriptional initiation site (Conway, et. al., 1991). The same is true for the *pgi* gene (Hesman, et. al., 1991). The *eno* promoter was also mapped (Burnett, et al., submitted for publication). The *glf* operon promoter region was found to share significant identity to the *eda, pgi, eno,* and several other highly expressed *Z. mobilis* promoters, but not to consensus promoters from other bacteria. The highly expressed *Z. mobilis* promoter set contains two independent, overlapping, conserved sequences that extend from approximately -100 to +15 with respect to the transcriptional start sites (Barnell, et. al., 1992). It is quite possible, perhaps even likely, that the structures of these highly expressed promoters identify them as members of a separate expression class that is characterized by high transcription rate.

4. Gene structure. One of the primary aims of this work has been to identify features of gene structure in *Z. mobilis* that facilitate high level expression of the glycolytic enzymes. High level expression of the genes can be attributed, in part, to very efficient translational initiation due to the high quality of the ribosome binding sites. A comparison of the highly expressed *Z. mobilis* genes indicated that the relative quality of the ribosome binding sites of these genes might play an important role in determining the relative level of enzyme synthesis (Conway, et. al., 1991). Interestingly, the *eno* gene does not have a strong ribosome binding site (Burnett, et al., submitted for publication), despite being the most abundant protein in *Z. mobilis* (Mejia, et. al., submitted for publication).

E. Specific Objective 3.

Progress in the area of metabolic flux control in *Z. mobilis* has been particularly challenging. The original proposal, to investigate the effects of genetically engineered alterations in specific enzyme levels on glycolytic flux, has been complicated in some rather interesting ways. First, two of the genes that encode enzymes that are thought to catalyze important flux-generating steps, glucose-6-phosphate dehydrogenase and glucokinase, are now known to be located in a four gene operon that is subject to a fascinating array of expression controls. Manipulation of *zwf* and *glk* expression must be independent of expression of each other and the other two genes in the operon. Second, two limitations of the originally proposed approach have been recognized and must be dealt with. In order to effectively evaluate the contribution of a specific enzyme to flux through a pathway, the level of that enzyme must be titrated, in vivo, both up and down in abundance. This is not possible with existing *Z. mobilis* experimental systems. We are currently testing two titratable expression systems in recombinant *Z. mobilis,* one based on the *tac* promoter and the enteric *lacI* gene, the second based on T7 promoter and polymerase regulated expression. Either of these systems will allow us to place expression of the *Z. mobilis* glycolytic genes under control of an exogenous inducer (IPTG). We are also examining two approaches for decreasing the level of specific enzymes. Both of these approaches involve the titratable promoter system, either by replacement of the genomic copy of the gene with the titratable promoter::gene fusion or by titrating antisense RNA expression. The second limitation involves experimental analysis of the in vivo effects of engineered changes in enzyme concentrations. It is possible to measure the absolute amounts of the enzyme levels by biochemical assay, immunoprecipitation, or by two-dimensional gel electrophoresis. However, these measurements say nothing about what is happening in vivo, which can only be appreciated by measuring the concentrations of the glycolytic intermediates in the cell pool. For this purpose, we have developed a sensitive, one-step HPLC procedure for analyzing the phosphorylated metabolic intermediate concentrations (see below). Of particular importance for the renewal application will be the effects of varying glucokinase and fructokinase levels.

F. Specific Objective 4.

1. Transcriptional regulation. Expression of *pgi, frk,* and the *glf* operon have been

found to be subject to carbon source-dependent regulation. The remainder of the *Z. mobilis* genes that have been characterized to date, *eda*, *gap-pgk*, *eno*, *adhB*, and *pdc* do not show carbon source-dependent regulation. The level of the 1.8 kb *pgi* transcript was found to be influenced by the phase of growth and by the carbon and energy source (Hesman, et. al., 1991). Transcript levels increased with respect to total RNA during logarithmic growth and were 3-fold higher when growing on fructose as compared to glucose. The level of the full-length 6.14 kb *glf* operon message was three-fold higher in cells grown on fructose as compared to cells grown on glucose (Barnell, et. al., 1992). Likewise, the 1.2 kb *frk* transcript was elevated more than two-fold when growing on fructose (Zembrzuski, et. al., 1992). In each case, enzyme levels paralleled mRNA levels. This increase was not the result of differential mRNA processing when grown on the different carbon sources, since the patterns of mRNA bands on northerns was similar for RNAs prepared from either glucose- or fructose-grown cells. Nor was it the result of differential transcript stability, since the degradation rates for each of the mRNAs was similar on both carbohydrates. Thus, the higher level of these mRNAs in fructose-grown cells must be the result of an increased rate of transcription from the three respective promoters. This has been confirmed for the *glf* promoter by use of *lacZ* fusions in recombinant *Z. mobilis* strains (unpublished data). The physiological role of increasing phosphoglucose isomerase and fructokinase activities is to increase flux of fructose into the pathway. The increase in *glf* expression when growing on fructose could be explained if the same transporter is used for both glucose and fructose transport. The affinity of the transporter is significantly greater for glucose than for fructose, perhaps necessitating increased *glf* expression in order to support the observed flux on fructose. The possibility that *glf* expression is responsive to fructose is further supported by the observation that the level of the *glf*-specific processed transcript is higher for cells grown on fructose, as judged by the ratio of the full length operon mRNA to the *glf*-specific mRNA (see Fig. 3 of Barnell, et al., 1992). It is reasonable to suggest that the transcriptional terminator-like structure downstream of *glf* and possible alternative secondary structures in the region are involved in *glf* regulation (see Appendix 2 and below).

Other aspects of transcriptional regulation, such as the participation of DNA binding proteins, are being assessed by so called "promoter titration" experiments. This work involves examining the effects of having multiple copies of the various promoters on expression of the intact, genomic copies of the genes. These experiments are being conducted in conjunction with efforts to genetically manipulate the levels of the respective enzymes in recombinant *Z. mobilis* strains.

2. Relative transcript stability. It is likely that high level expression of the glycolytic genes is coordinated at the level of mRNA abundance. The relative abundance of specific mRNAs is regulated by the relative rates of transcription and message decay. In a collaborative study with Dr. L. O. Ingram, transcript stability and the abundance of the gene products have been determined for eight of the glycolytic enzymes (Mejia, et. al., submitted for publication). The transcripts were all unusually stable, with half-lives ranging between 8 and 18 minutes. Transcript stability is proposed to be a dominant feature in distinguishing between the highly expressed glycolytic genes and the vast majority of poorly expressed biosynthetic and house keeping genes. Among the eight glycolytic genes studied, differences in transcript stability were found to correlate directly with protein levels. Thus, differential transcript stability is hypothesized to be a primary mechanism for balancing the levels of individual enzymes of the *Z. mobilis* glycolytic pathway.

3. Post-transcriptional regulation. The full-length 6.14 kb polycistronic *glf-zwf-edd-glk* mRNA from *Zymomonas mobilis* appears to be processed by endonucleolytic cleavage, resulting in the formation of several discrete transcripts (Liu, et. al., 1992). Northern analysis and transcript mapping revealed that the processed transcripts correspond to functional mono-, di-, or tri-cistronic messages. The relative abundance of the gene-specific, functional messages was measured. Expression of *zwf* and *edd* correlated well with functional message levels. Dispropor-

tionally high levels of the *glk*-specific mRNAs might compensate for the instability of glucokinase by allowing increased translation. The relative abundance of the discrete transcripts was shown to be a function of their respective decay rates. Northern analysis of the fate of the 6.14 kb transcript following inhibition of transcription by rifampicin showed that the abundance of shorter, more stable transcripts increased at the expense of longer, less stable transcripts. This is suggestive of endonucleolytic mRNA processing. The most abundant 5' and 3' transcript ends were found to lie within secondary structures that probably impart stability to the most abundant mRNAs. The most complicated region of secondary structure in the *glf* operon lies in the intergenic region immediately downstream of *glf*. This region contains a possible transcriptional terminator that could be obscured by formation of other, larger secondary structures (Appendix 2). The role of these complicated secondary structures in balancing synthesis of the glucose facilitator with the subsequent metabolic enzymes and the exact chronology of the mRNA processing events remain to be elucidated.

4. Relative glycolytic gene expression. The genetic mechanisms by which high level synthesis of the Entner-Doudoroff glycolytic pathway enzymes are balanced in *Z. mobilis* are beginning to be understood. A growing body of evidence indicates that several layers of genetic control, perhaps in a hierarchal arrangement, act in concert to determine the relative abundance of the enzymes. The genes all possess canonical ribosome binding sites and display high codon bias. Co-translation of the overlapping *zwf* and *edd* genes might be important for controlling the balance of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydratase. The relative stabilities of the mRNAs appear to play a role in coordinating expression of the glycolytic genes. Likewise, mRNA processing appears to be important for dictating an appropriate balance of the glycolytic enzymes. Promoter strength and the relative rates of transcription of the glycolytic genes, which appear to form a separate, high expression class, are likely to be important. One further level of control might be imposed by modulation of promoter activity by transcription factors.

G. Characterization of the *Z. mobilis* Glucose Facilitator

The fortuitous cloning of the glucose facilitated diffusion transporter, *glf*, is a real stroke of luck. This is the only fully accepted example of a facilitator system in the prokaryotic world. Genetic reconstitution of glucose transport in *E. coli* mutants has been used as a preliminary means for confirming biochemical function of the *Z. mobilis* glucose facilitator. The *glf* gene was transformed into *E. coli* JM1100 (*ptsG ptsM ptsF galP*), a strain that is genetically defective in glucose transport and unable to grow on minimal glucose media. *E. coli* JM1100 (pTC111, glf^+) is able to grow with a generation time approaching that of an isogenic *E. coli* strain that is $ptsG^+$. *E. coli* JM1100 (pTC111) is also able to grow on fructose, albeit more slowly, suggesting that *glf* encodes a facilitator that functions for transport of both glucose and fructose. Complementation of growth on glucose by *E. coli* ZSC113 (*ptsG ptsM glk*) required expression of both the *Z. mobilis glf* and *glk* genes.

Given that yeast and other higher organisms possess several different glucose facilitators, it seems reasonable to search for *glf* homologs in *Z. mobilis*. A *Z. mobilis* expression library was transformed into *E. coli* JM1100 and plated onto glucose-MacConkey and fructose-MacConkey agar. Presumably, any clones with restored ability to ferment carbohydrates would have received a *Z. mobilis* gene that allowed carbohydrate transport. A number of clones capable of fermenting glucose or fructose were obtained. The glucose-fermenting clones were screened for the ability to ferment fructose and vice-versa. Most of the clones were able to ferment both carbohydrates, but interestingly, several clones could ferment glucose and not fructose while a single clone was able to ferment fructose and not glucose. Restriction digests of the library clones indicated several were identical to *glf*. Other library clones appear, on the basis of restriction mapping, to be distinct genes. Characterization of a few of these presumptive transport genes would be a worthwhile project that could be conducted by an undergraduate student.

H. Additional Studies Not Within the Original Purview of Current Grant

1. HPLC method development for metabolite analysis. Relative contributions of individual enzyme steps can in theory be assessed by application of the Metabolic Control theory, yet the actual analysis of carbon flux control is quite involved when working with in vivo systems. It is clear from Metabolic Control theory that the enzymes and their properties control fluxes and that intermediary metabolite concentrations adjust themselves to their steady state values as a consequence of these properties. Therefore, flux control coefficients can be calculated for an in vivo system under conditions in which metabolite concentrations can be measured. Traditional approaches for measuring metabolite pools have relied upon cumbersome and time consuming enzyme titration experiments or upon NMR analyses with flow cells connected to chemostats which make the examination of more than a few cultures difficult. For this reason, we have developed a rapid, sensitive HPLC method for measuring metabolite concentrations in cell pool extracts.

The top HPLC trace in Appendix 1 shows the separation by anion exchange and detection by suppressed conductivity of 25 nanomole injections of several metabolites in a single run. The identified compounds that have been detected include nearly all of the three- and six-carbon phosphorylated glycolytic intermediates, all of the nucleotide mono-, di-, and tri-phosphates, and many of the carboxylic acid intermediates of the TCA cycle. For measurement of in vivo metabolite concentrations, the method basically involves growing cells in batch cultures, followed by rapid filtration and boiling water extraction of the cell pool compounds, the results of which are shown in the lower HPLC trace in Appendix 1. Quantitation is performed by computer and concentration is determined based upon the known intracellular volume of *Z. mobilis*. We have identified nearly 30 different compounds in *E. coli* extracts by co-chromatography and have established that the measured metabolite concentrations reflect the metabolic state by comparing starved and metabolizing cells. The HPLC methodology is essentially developed to the point where it can be utilized to help fulfill specific objective 3 of the current proposal and can be used for the experiments outlined in the renewal proposal.

2. The Entner-Doudoroff pathway in *E. Coli*. In *Escherichia coli* the two enzymes of the Entner-Doudoroff pathway are strictly inducible for gluconate catabolism. Individually, KDPG aldolase is also a component of another pathway for hexuronic acid metabolism. Using the cloned *edd* and *eda* genes as tools, we examined transcriptional expression of the Entner-Doudoroff pathway (Egan, et al., 1992). Induction by growth in the presence of gluconate leads to synthesis of a 2.6 kb message that is of sufficient length to encode both genes and hybridizes to both gene-specific probes. Thus, the genes of the Entner-Doudoroff pathway form an operon, in the order *edd-eda*. High basal aldolase activity is explained by constitutive transcription of two *eda*-specific messages, with sizes of 1.0 and 0.75 kb. Modest increases in the level of the 0.75 kb transcript were observed on media containing gluconate, while increased levels of the 1.0 kb transcript were observed on media containing hexuronic acids.

The Entner-Doudoroff pathway was predicted to be induced for oxidative glucose metabolism when cultures were provided with the periplasmic glucose dehydrogenase cofactor, pyrroloquinoline quinone (PQQ; Adamowicz, et al., 1991). More recently, we have established on the basis of biochemical and molecular genetic analysis that this pathway is functional in glucose transport mutants as well as wild type strains of *E. coli* (unpublished data). These data complete the body of evidence that prove the existence of a pathway for oxidative glucose metabolism in *E. coli*. PQQ-dependent oxidative glucose metabolism provides a metabolic branch point, *in the periplasm*; the choices being either oxidation to gluconate followed by induction of the Entner-Doudoroff pathway or phosphotransferase-mediated transport. The oxidative glucose pathway might be important for survival of enteric bacteria in aerobic, low phosphate, aquatic environments.

The nucleotide sequence of the entire *E. coli edd-eda* region that encodes the enzymes of the Entner-Doudoroff pathway was determined (Egan, et al., 1992). The *edd* structural gene begins 236 bases downstream of *zwf*. The *eda* structural gene begins 34 bases downstream of *edd*. The *edd* reading frame is 1809 bases long and encodes the 602 amino acid, 64,446 dalton protein, 6-phosphogluconate dehydratase. The deduced primary amino acid sequences of the *E. coli* and *Z. mobilis* dehydratase enzymes are highly conserved. The *eda* reading frame is 642 bases long and encodes the 213 amino acid, 22,283 dalton protein, 2-keto-3-deoxy-6-phosphogluconate aldolase. This enzyme had been previously purified and sequenced, by others, on the basis of its related enzyme activity, 2-keto-4-hydroxyglutarate aldolase. The data presented here provide proof that the two enzymes are identical. The primary amino acid sequences of the *E. coli*, *Z. mobilis*, and *P. putida* aldolase enzymes are highly conserved. When *E. coli* is grown on gluconate, the *edd* and *eda* genes are cotranscribed. Four putative promoters within the *edd-eda* region were identified by transcript mapping and computer analysis. P_1, located upstream of *edd*, appears to be the primary gluconate-responsive promoter of the *edd-eda* operon, responsible for induction of the Entner-Doudoroff pathway, as mediated by the *gntR* product. High basal expression of *eda* is explained by constitutive transcription from P_2, P_3, and/or P_4, but not P_1.

I. Continued Work During this Funding Period.

At this time, the research plans remain intact relative to the original proposal. To date, the first two of four specific objectives have been achieved and the fourth objective is virtually complete. During the third and final year of the current funding period, the majority of effort will be devoted to fulfillment of the third specific objective, an investigation of potentially rate-controlling steps in glycolytic metabolism. *Z. mobilis* has proven to be a most interesting organism and gives every indication of harboring additional secrets that are worth pursuing.

J. Refereed Publications Supported by DOE

Burnett, M. E., J. Liu, and T. Conway. 1992. Molecular characterization of the *Zymomonas mobilis* enolase (*eno*) gene. (Submitted to J. Bacteriol.)

Conway, T. 1992. The Entner-Doudoroff pathway: History, physiology, and molecular biology. FEMS Microbiological Reviews. (Invited review; IN PRESS)

Shark, K. and T. Conway. 1992. Cloning and molecular characterization of the DNA ligase gene (*lig*) from *Zymomonas mobilis*. FEMS Microbiol. Letts. (IN PRESS)

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Mejia, J.P., M.E. Burnett, H. An, W.O. Barnell, K.F. Keshav, T. Conway and L.O. Ingram. 1992. Coordination of *Zymomonas mobilis* glycolytic and fermentative enzymes: A simple hypothesis based on mRNA stability. (submitted to J. Bacteriol.)

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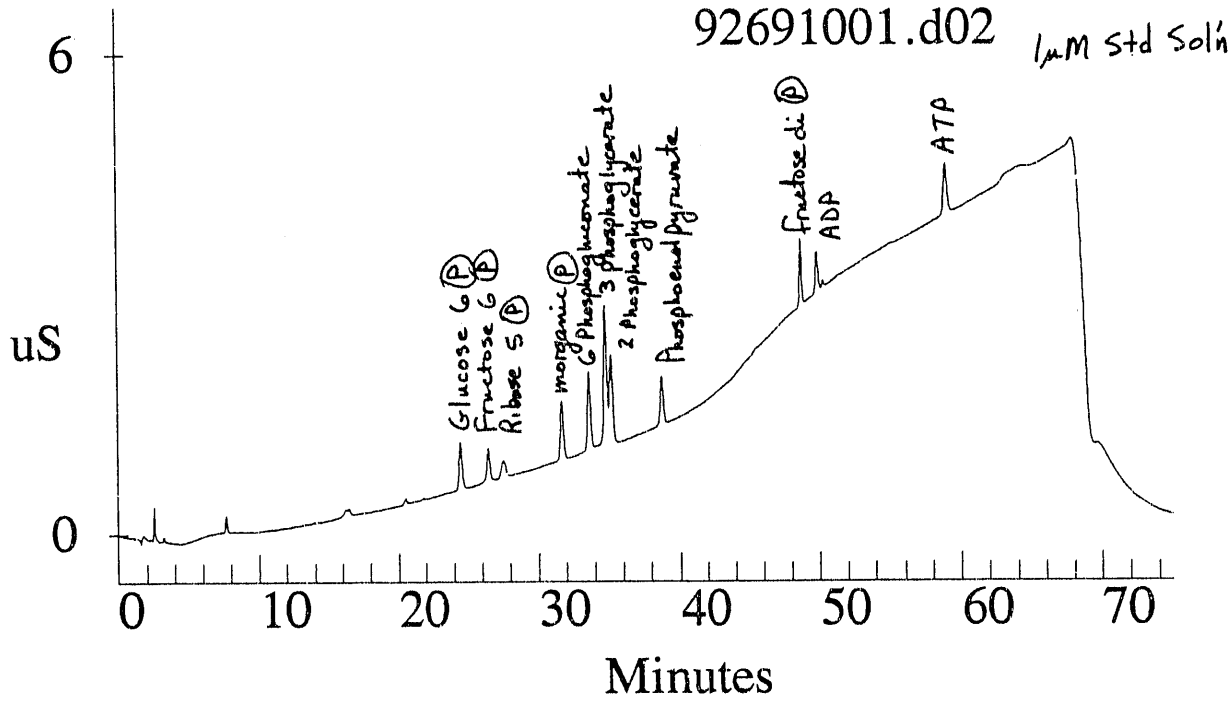
Hesman, T.L., W.O. Barnell and T. Conway. 1991. Cloning, characterization and sequence analysis of a phosphoglucose isomerase gene (*pgi*) from *Zymomonas mobilis* that is subject to carbon source-dependent regulation. J. Bacteriol. 173: 3215-3223.

*Barnell, W.O., K.C. Yi, and T. Conway. 1990. Sequence and genetic organization of a *Zymomonas mobilis* gene cluster that encodes several enzymes of glucose metabolism. J. Bacteriol. 172: 7227-7240.

[*Included in Progress Report]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Ribose 5 P
inorganic P
6 Phosphogluconate
3 Phosphoglycerate
2 Phosphoglycerate
Phosphoenol pyruvate
Fructose di P
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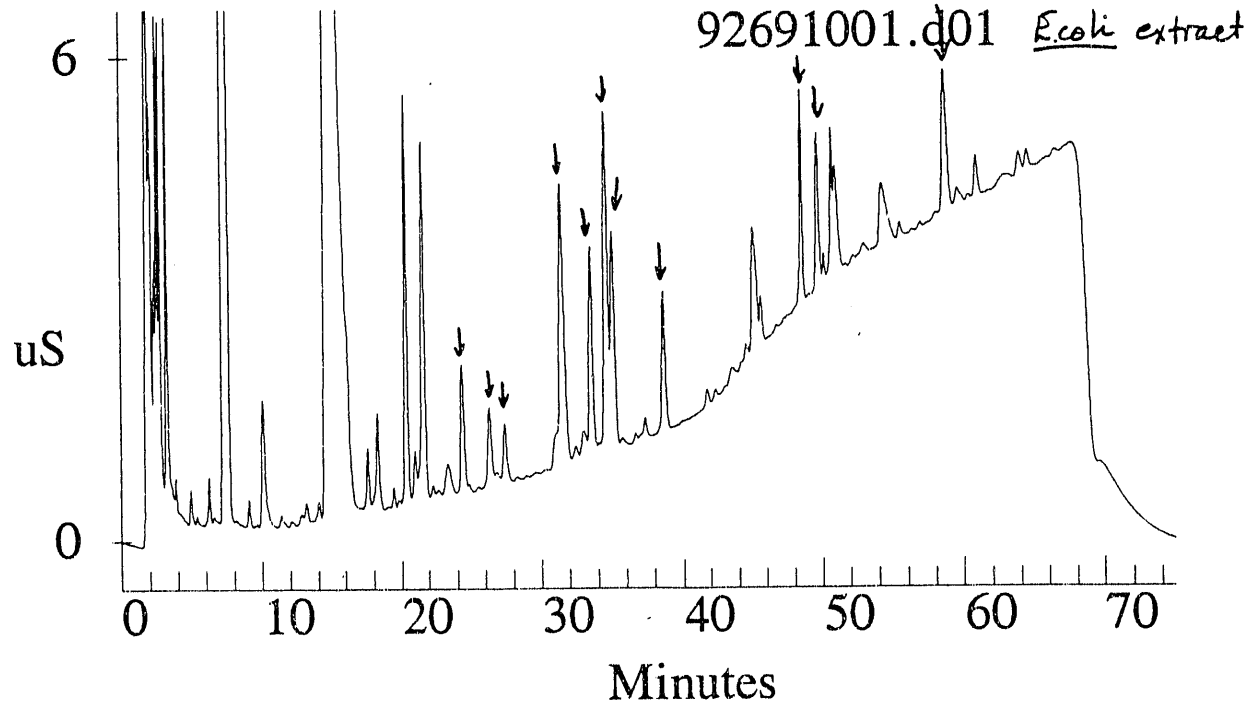
70
Minutes


92691001.d01
E.coli extract
6
0
uS
0
10
20
30
40
50
60
70
Minutes

END

DATE FILMED

9/17/92