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Regulation of Alcohol Fermentation by Escherichia coli

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The purpose of this project is to elucidate the way in which the synthesis of ethanol and related fermentation products are regulated in the facultative anaerobe Escherichia coli. We are also investigating the control of other genes required for anaerobic growth. We have isolated both structural and regulatory mutations affecting the expression of alcohol dehydrogenase, the enzyme responsible for the final step in alcohol synthesis. Some of these regulatory mutations also affect other anaerobically induced genes. The adh gene has been cloned and sequenced. The ADH protein is one of the largest highly expressed proteins in E. coli and requires approximately 2700bp of DNA for its coding sequence. We have also isolated mutations affecting the fermentative lactate dehydrogenase and have recently cloned the ldh gene. In consequence it is now possible to construct E. coli strains defective in the production of any one or more of their normal fermentation products (i.e. formate, acetate, lactate, ethanol and succinate). The factors affecting the ratio of fermentation products are being investigated by in vivo NMR spectroscopy. Examination of our collection of anaerobically controlled gene fusions has shown that many can be switched on in air upon treatment with cyanide or certain other inhibitory agents. We are investigating the basis for this effect at present.

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PROGRESS REPORT
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REGULATION OF ALCOHOL FERMENTATION IN ESCHERICHIA COLI

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This progress report includes the following manuscripts which are in various stages of completion. All are attributable to DOE funding. Some of the recently published papers were included in the form of "submitted manuscripts" in my previous report.

1. Mat-Jan, F., Williams, C.R. and D.P. Clark (1989). Anaerobic growth defects resulting from gene fusions affecting succinyl-CoA synthetase. *Molec. Gen. Genet.* 215: 276-280.
2. Gupta, S. and D.P. Clark (1989). Escherichia coli derivatives lacking both alcohol dehydrogenase and phosphotransacetylase grow anaerobically by lactate fermentation. *J. Bacteriol.* 171: 3650-3655.
3. Clark, D.P. (1989). The fermentation pathways of Escherichia coli. *FEMS Microbiology Reviews.* 63: 223-234.

4. Goodlove, P.E., Cunningham, P.R., Parker, J.M. and D.P. Clark (1989). Molecular cloning and sequence of the fermentative alcohol dehydrogenase of Escherichia coli. Gene. 85: 209-214.
5. Alam K.Y. and Clark D.P. (1989). Anaerobic fermentation balance of Escherichia coli as observed by in vivo nuclear magnetic resonance. J. Bacteriol. 171: 6213-6217.
6. Clark D.P. Molybdenum cofactor negative mutants of Escherichia coli use citrate anaerobically. FEMS Microbiol. Letts. In Press.

The review (#3 above) summarizes much of our work over the last few years and includes a brief description of some recent, unpublished observations.

A. The adhE gene sequence

In collaboration with Dr. J. Parker of this department we have sequenced the adhE gene (Goodlove et al, Ref 4 above). A sequence of 2673 bases coding for a protein of 891 amino acids was found between positions 1035 and 3708 of the sequenced region of the adhE plasmid pHIL8. Within this sequence an inverse open reading frame was found which codes for a possible protein of just over 400 amino acids. The regulatory sequences for anaerobic regulation of the adhE gene are not on plasmid pHIL8.

We have therefore obtained a λ phage carrying the adhE region of the E. coli chromosome. We have subcloned from this a DNA segment carrying adhE gene which does have the correct anaerobic

regulatory sequences attached to it. ADH from our new series of plasmids is regulated correctly. Recently we have subcloned the regulatory region for adhE into a chloramphenicol fusion vector to give a gene fusion in which the chloramphenicol resistance gene is regulated by the adh control sequences. Further analysis of the adh control region is in progress.

B. The lactate dehydrogenase (ldhA) gene.

We previously isolated mutants defective in the fermentative lactate dehydrogenase and mapped these to 30.4 min on the *E. coli* chromosome. As a result we were able to identify the λ phage from the physical map collection which carries the ldhA gene. The ldhA gene has been subcloned on a PstI-BamHI fragment of just over 4 kilobases. We will be investigating the regulation of this gene and characterizing its protein product in more detail.

C. Fermentation products.

We have used in vivo NMR spectroscopy of anaerobic cultures to analyze the mixture of fermentation products. A series of mutants affecting fermentation and constructed strains carrying several mutations have been surveyed (see manuscripts #2, 3 and 5). As expected mutations in adhE abolish the production of ethanol, those in ldhA abolish lactate and pta/ack mutants lack acetic acid. More interestingly double mutants in adhE and pta produce no ethanol or acetate but do grow anaerobically on glucose by what is essentially a homolactic fermentation (see #2). The critical issue (discussed in detail in my review #3) is the question of redox balance. Reducing

equivalents produced during glycolysis must be consumed during fermentation. The proportions of ethanol (highly reduced) lactate (medium reduced) and acetate (no consumption of reducing equivalents) vary in response to the level of reduction of the fermentation substrate. With sugar alcohols e.g. sorbitol, which are more reduced than glucose, the proportion of ethanol is higher whereas with sugar acids (e.g. gluconic acid, glucuronic acid) the amount of ethanol is less than for glucose. We have confirmed that the hydrogen released from formate by formate hydrogen lyase may be recycled and used to increase the yield of ethanol and succinate under some conditions (see #5).

We have recently discovered that several ubiquinone negative mutants of E. coli lack acetaldehyde dehydrogenase activity (but retain alcohol dehydrogenase activity). It seems plausible that transfer of extra reducing equivalents to ethanol may involve a quinone linking the membrane electron transport systems with the enzymes of fermentation. This area deserves future investigation.

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