STUDIES OF GENE EXPRESSION FROM THE *MET* B PROMOTOR OF *ESCHERICHIA COLI*: ATTEMPTS TO ISOLATE GENE FUSION PRODUCTS

by

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ABSTRACT

Attempts were made to study the regulation of the met BJLMF gene cluster in Escherichia coli by such techniques as gene fusion and deletion analysis. Due to technical difficulties the gene fusion technique was abandoned. However, a procedure for the isolation of deletion mutants based on the technique of Parkinson and Huskey (J. Mol. Biol. 1971. 56: 369-384.) was established. Using this procedure, presumptive deletions carrying portions of the met BJLMF region have been isolated.

ACKNOWLEDGEMENTS

Many thanks to Dr. James R. Johnson for his patience and guidance and for the use of his laboratory during my research.

To Cheryl Lilgestrand, I appreciate the use of your bacteriophage, lambda dmet 145.

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The biosynthetic pathway of the essential amino acid methionine in Escherichia coli is complex, multi-branched, and to this date it's regulation remains only partially characterized. Enzymes governing methionine production are coded by genes dispersed across the E. coli K-12 chromosome but primarily situated at 87 minutes (figure 1). Our research concerned the met J loci which is comprised of the genes met B, met J, met LM, and met F coding respectively for the following proteins: cystathionine gamma-synthase, repressor protein, aspartokinase 2 and homoserine dehydrogenase 2, N5-N10 methyline tetrahydrofolate reductase. (8, 9, 10)

Two methods for the study of the regulation of methionine biosynthesis were undertaken in this research. Originally, a procedure developed by Malcolm Casadaban in 1975 was attempted in which the met B promotor was to be fused to the Z gene of the already fully characterized lactose operon. This structural gene codes for the easily assayable enzyme Beta-galactosidase. (1, 4) The Casadaban technique involves three basic steps.

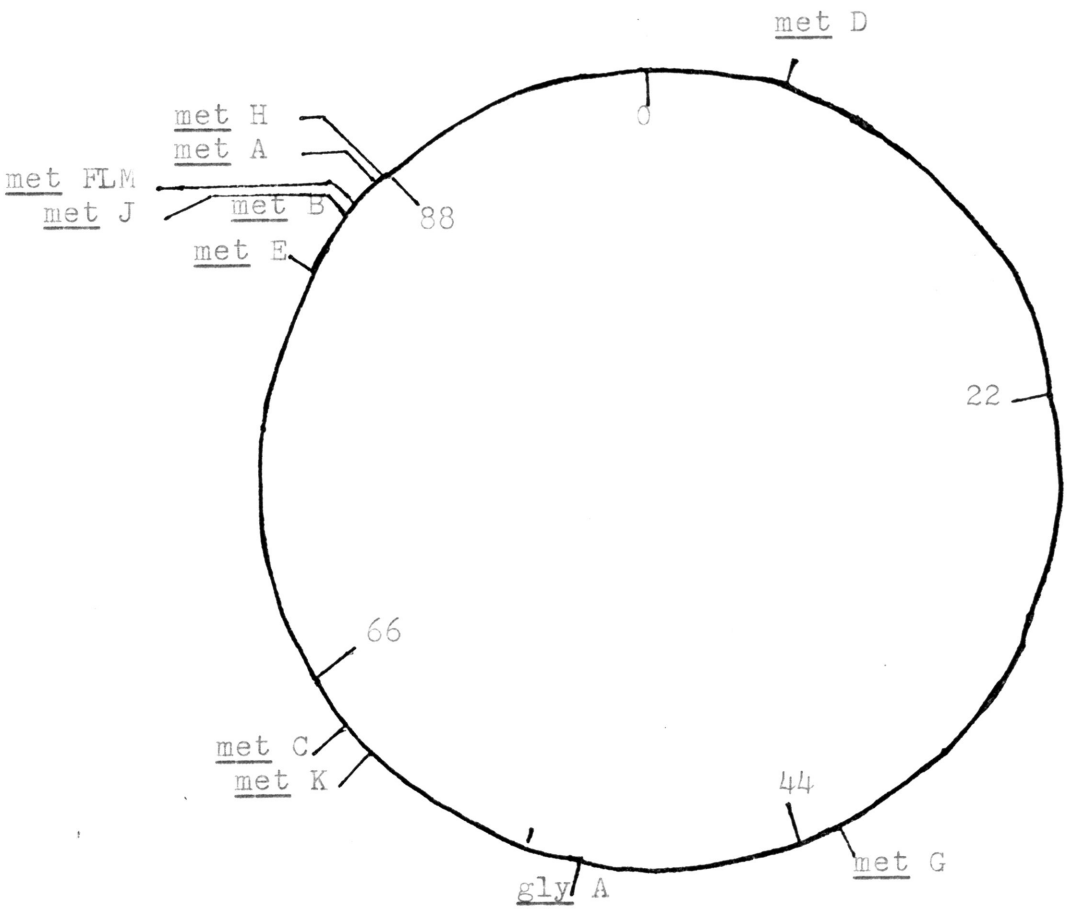
1. Transduction between two laboratory strains of E. coli to produce a desired lambda attachment and lactose operon deleted cell strain (2, 3)

2. Mutagenesis of the met B gene with a Mu temperature

*The format for this paper follows that of the Journal of Bacteriology, 1978.

Figure 1

POSITION OF THE MET REGULON GENES
ON THE E. COLI CHROMOSOME



sensitive repressor strain carrying an ampicillin resistance transposon, followed by integration of a lambda-lac-mu hybrid transducing phage into the mutagenized gene (5, 7, 14)

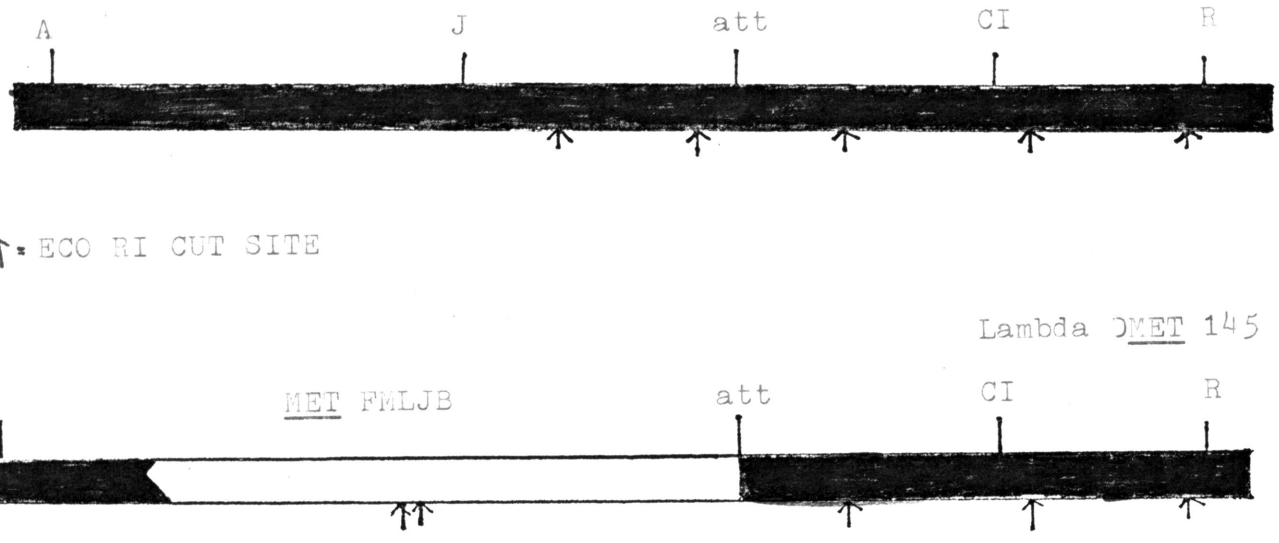
3. Isolation of recombinant lambda-met-lac-mu hybrid transducing phages from the constructed cell strain Although this technique has been successfully utilized in the study of the regulation of gene expression in the arabinose operon, leucine operon, isoleucine valine operon, and arginine regulon (6, 13), due to technical difficulties, it proved to be too time consuming to complete for the methionine system in the allowed six months. For this reason, the Casadaban procedure was abandoned and replaced by a procedure for deletion analysis of a cloned met gene cluster (12).

MATERIALS AND METHODS

Materials. The chelating agent ethylenedinitrilo-tetraacetic acid (EDTA) was prepared at a concentration of .01M in 0.01M Tris HCL pH=7.4. The bacterial strain JJ125, which has mutations in the met B and met F genes, served as the recipient cell in the transductions. Bacteriophage lambda dmet 145, a defective phage which carries a segment of bacterial chromosome on which the met FMLJB genes (figure 2) are situated was donated by Cheryl Lilgestrand. (9, 10) The bacteriophage lambda vir which has mutations in the left and right operators as well as a mutation inactivating the repressor gene was

FIGURE 2

STRUCTURE OF THE LAMBDA DMET 145 PHAGE



available in the laboratory. Bacteriophage lambda b2c which carries a mutation inactivating it's repressor gene and a deletion removing one-half to three-fourths of it's phage attachment site was also available in the laboratory.

EDTA Inactivation of Lambda dmet 145. Inactivation of bacteriophages lambda dmet 145 (defective transducing phage), lambda c1857amS7 (helper), and lambda b2c (control) was conducted at both 42 degrees Centigrade and 55 degrees Centigrade. A 1:10, 1:50, and 1:100 dilution of the phage lysates were inactivated at each of the above temperatures. The actual procedure entailed addition of diluted phage to a pre-warmed flask of EDTA at a concentration of .01M with subsequent removal of increments at 0, 5, 10, 15, 30, and 45 minutes. The samples were immediately added to .01M MgSO4 (final concentration) and placed in an ice water bath to halt the inactivation.

Testing for Plaque-forming Activity. Plaque-forming activity tests were conducted to titer the remaining viable bacteriophages in each sample. The bacterial cell RW262 was overlayed on LB-lambda nutrient agar. Decreasing serial dilutions of each sample were then spotted on the agar overlay and incubated at 37 degrees Centigrade. At this temperature bacteriophage lambda undergoes a lytic cycle, thus, the result appears as a solid lawn of bacterial cells with clear plaques where the viable viruses were spotted.

Testing for Transducing Activity. Transduction is the viral mediated transfer of bacterial genetic information within cells. Each sample of the virus lambda dmet 145 was tested for it's ability to transfer the met F and met B genes to the bacterial cell JJ125 through lysogenization. Starved JJ125 cells were spread on minimal media both supplemented and unsupplemented with d,l cystathionine. Serial dilurions of each phage sample were then spotted on the plates and incubated at 32 degrees Centigrade. As lambda forms lysogens at this temperature, only those cells infected by lambda dmet 145 were able to grow.

Plate Transduction. In order to isolate individual lysogens, several plate transductions were made. In this procedure, starved JJ125 cells were infected at 32 degrees Centigrade with an equal volume of EDTA treated lambda dmet 145. The cells were then concentrated, diluted, and spread on d,l cystathionine agar. This supplement allows for selection of JJ125 transductants lacking met B function. Hopefully most of these transductants would be lysogens lacking the met B gene on the lambda dmet 145 chromosome. Colonies which grew rapidly were selected and plated on minimal medium. Those colonies which grew on minimal medium were discarded as presumptive normal lambda dmet 145 lysogens. Those not growing on minimal medium were further tested.

Immunity Test. The immunity test determines whether or not a cell is a lysogen. In this procedure, presumptive

lysogens are streaked across the viruses lambda vir and lambda b2c. If lambda vir lyses the cells but lambda b2c does not, it indicates that they are in fact lysogens. (11)

RESULTS

The deletion analysis procedure, outlined by Parkinson and Huskey in 1970, involves the EDTA selection of bacteriophages from which chromosomal segments have been deleted. EDTA, or ethylenedinitrilotetraacetic acid, is a chelating agent which causes the rupture of proteinaceous viral capsids. This inactivation is dependant upon the following variables: 1) The density of the bacteriophage 2) The length of exposure to EDTA 3) The temperature during EDTA exposure 4) The quantity of bacteriophage present in the initial sample (12)

With respect to density, the more dense the bacteriophage, the more DNA is present, and the more likely the phage is to rupture on exposure to a chelating agent. It has been shown by Parkinson and Huskey that the larger the deletion in a bacteriophage, the less dense the phage, and the less likely the phage is to be inactivated (figure 3). It should be noted that the procedure of Parkinson and Huskey utilized Sodium Pyrophosphate as a chelating agent rather than EDTA.

Inactivation is also dependent upon the length of exposure and the temperature of exposure. In general, the longer the exposure time, the greater the inactivation.

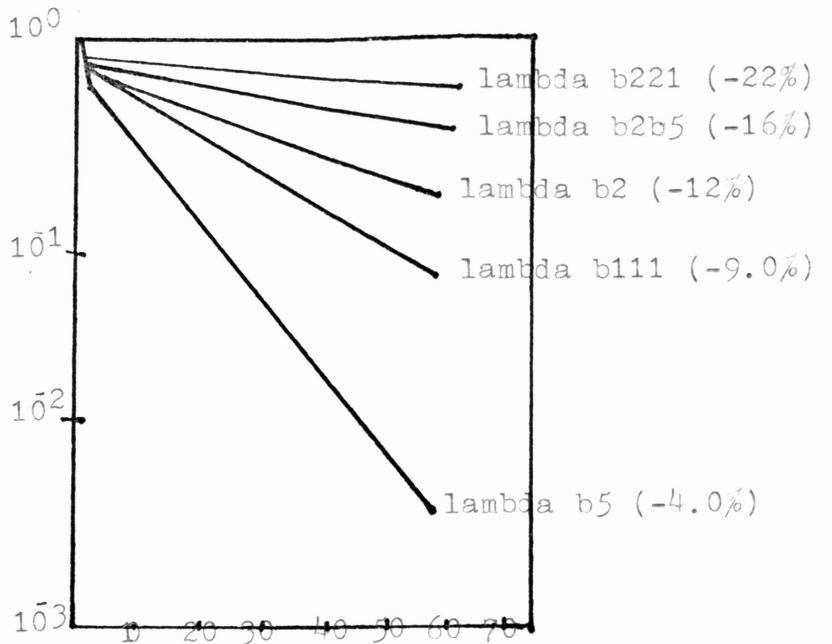


FIGURE 3: Parkinson and Huskey, J. Mol. Biol., 1970, 56:369-384.

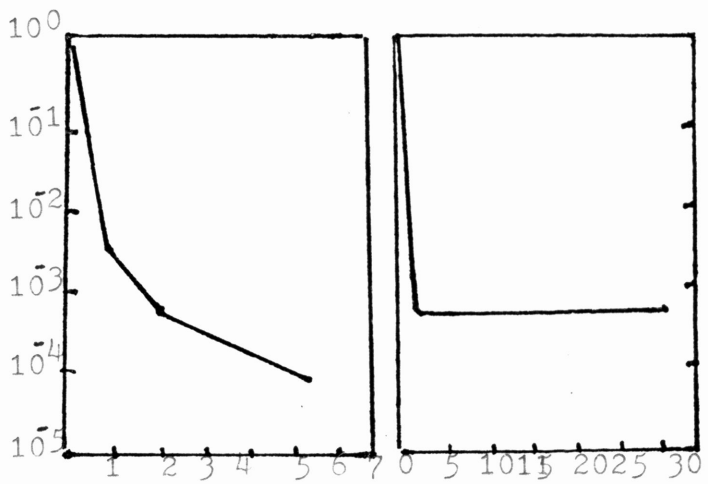


FIGURE 4: Parkinson and Huskey, J. Mol. Biol., 1970, 56: 369-384.

As illustrated in figure 4, however, the combination of chelating agent and an intermediate temperature yields a faster rate of inactivation than a gigh temperature alone. But, the higher temperature yields a greater total inactivation of the virus particles.

Finally, the quantity of bacteriophage present in the initial sample is important. At high concentrations of bacteriophage the .01M EDTA concentration is ineffective.

In order to carry out a selection for deletion mutants of lambda d<u>met</u> 145 in our laboratory it was first necessary to establish a protocol using the available equipment and EDTA as the chelating agent. Our results are illustrated in figures 5, 6, and 7. We ran the inactivation at 1:10, 1:50, and 1:100 dilutions of the original phage sample. We also performed the experiment at both 42 degrees Centigrade and 55 degrees Centigrade. As illustrated in figure 5, the control phage lambda b2c yielded it's greatest decreas in titer, approximately two logs, at 55 degrees Centigrade and at a 1:100 dilution. These conditions were selected as optimum. The helper phage lambda c1857amS7 showed a four log decrease in plaque-forming titer at 55 degrees Centigrade for a 1:a00 dilution (figure 6). In terms of it's transducing activity the defective phage lambda d<u>met</u> 145 yielded the most rapid decrease of the three for a 1:100 dilution at 55 degrees Centigrade (figure 7). These experimental results are in accordance with those of Parkinson

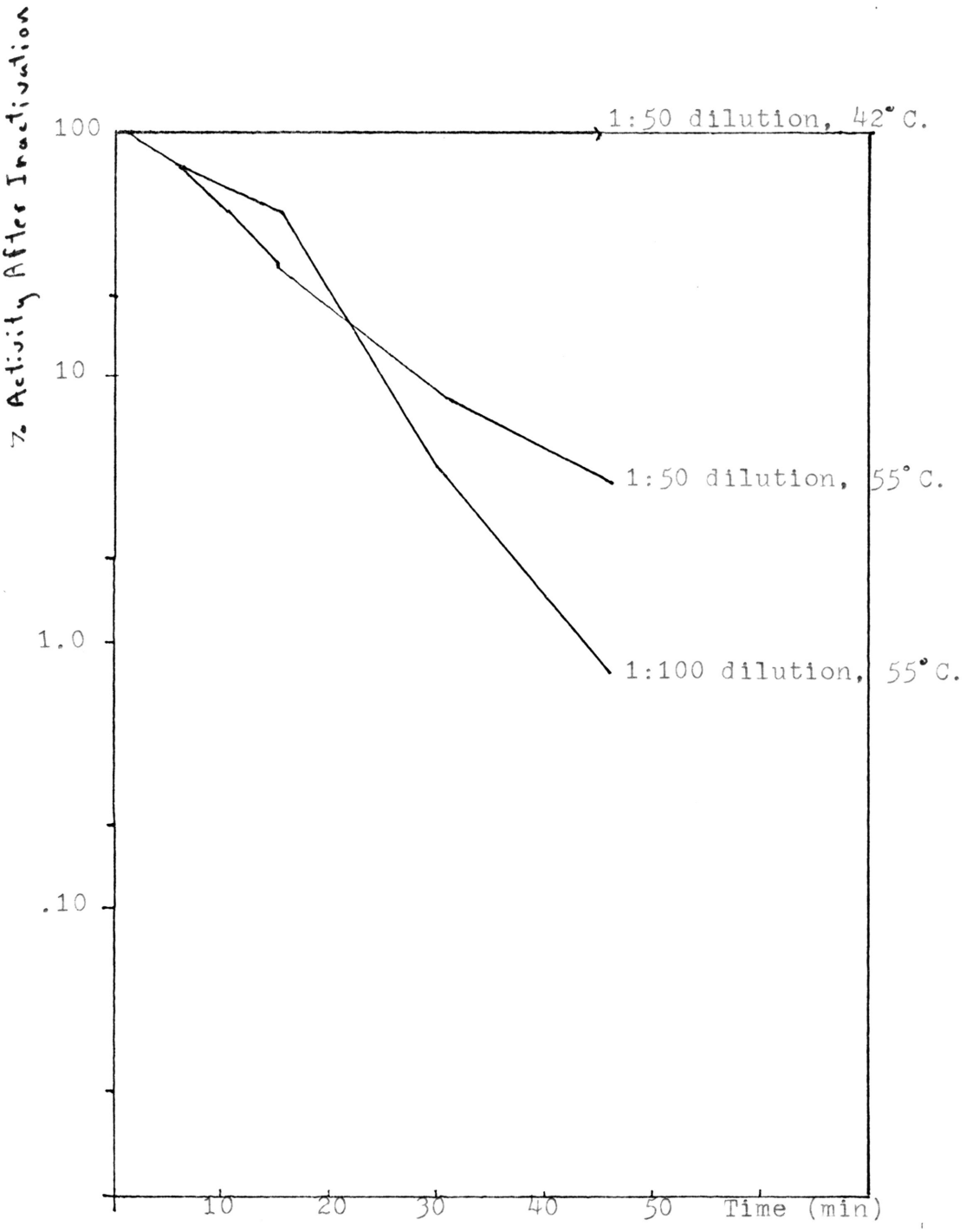


FIGURE 5: Lambda b2c Plaque-forming Activity

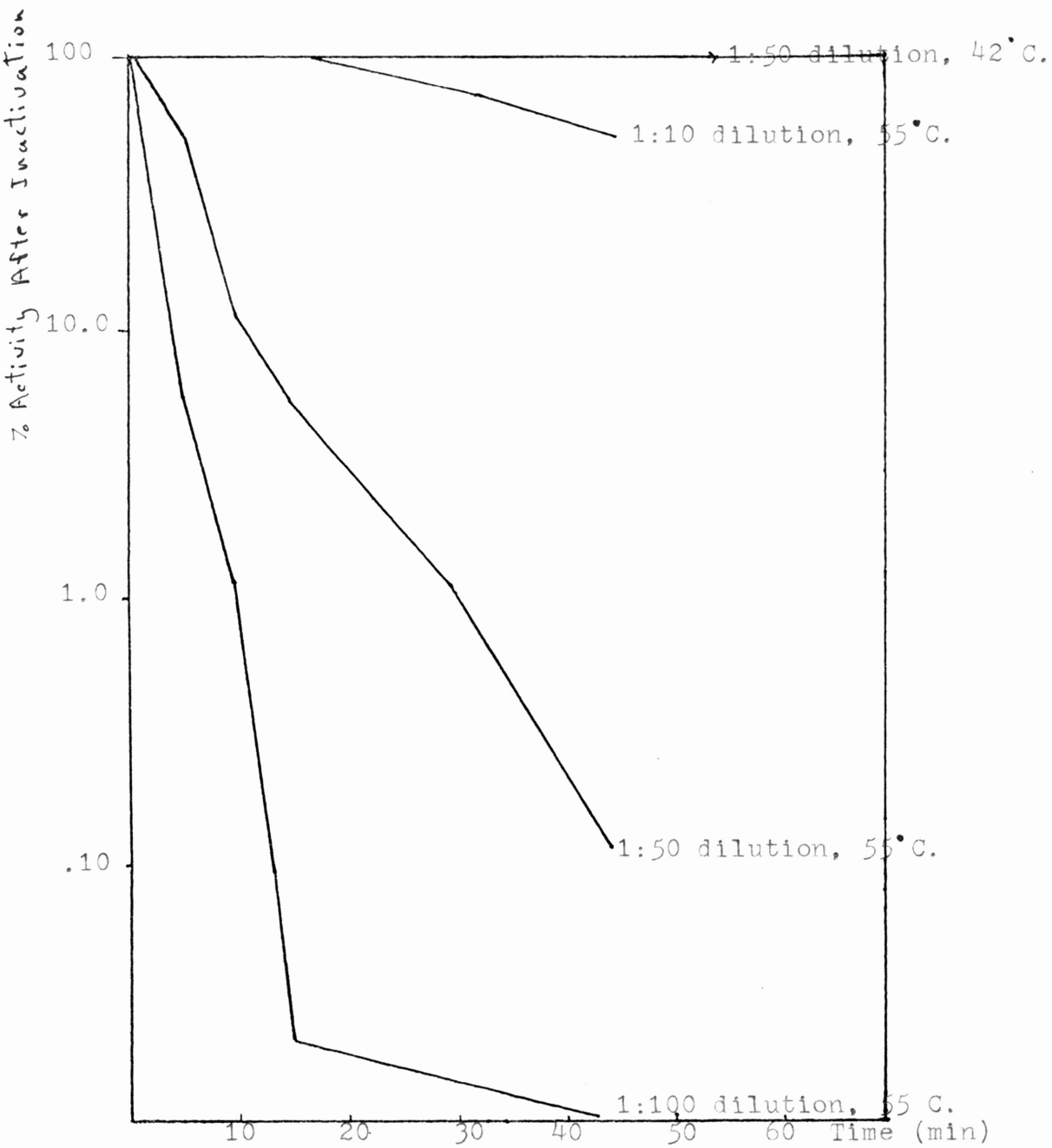


FIGURE 6: Lamba c1857amS7 Plaque-forming Activity

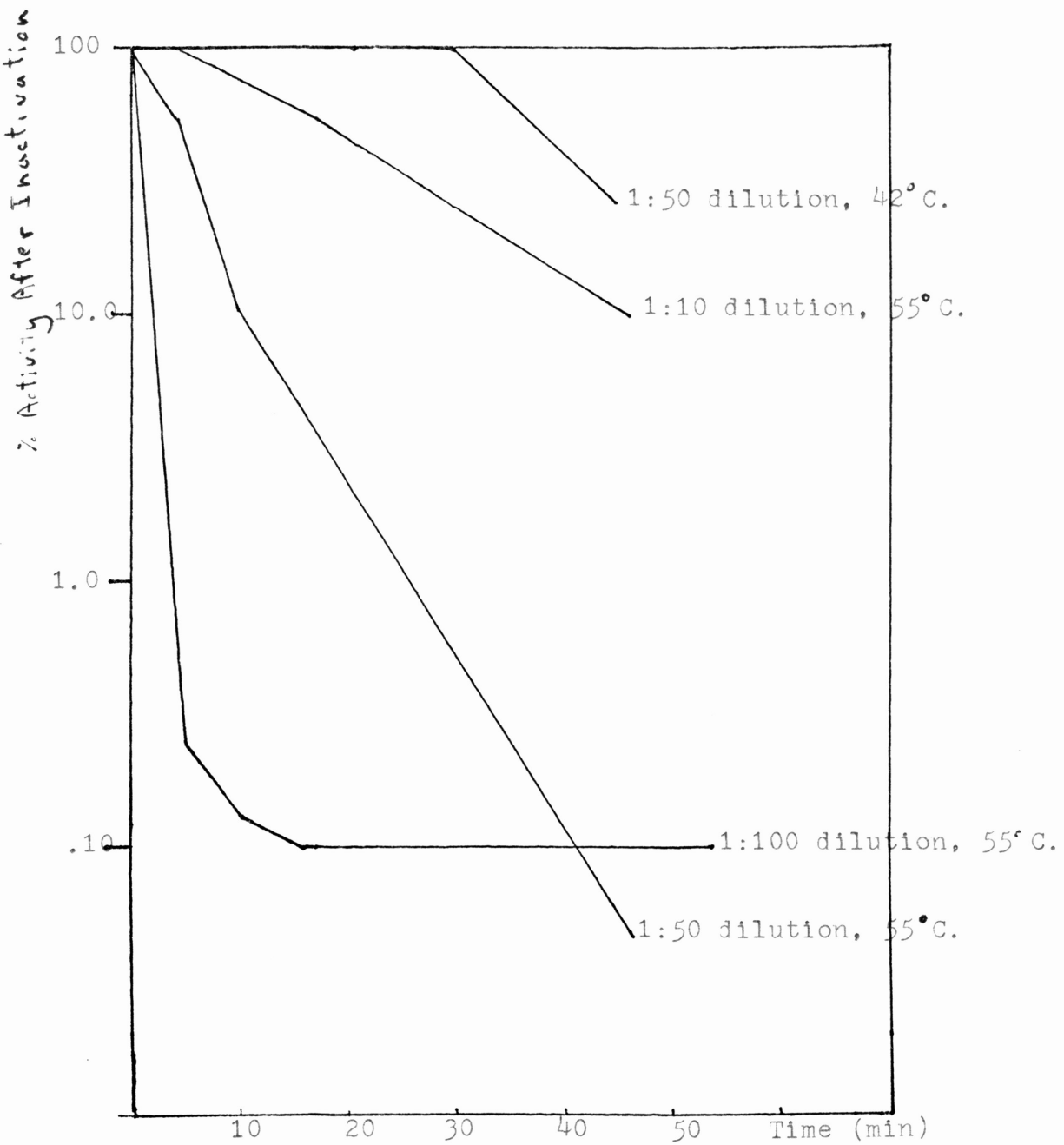


FIGURE 7: Lambda dmet 145 Transducing Activity

Huskey in figures 3 and 4. The control phage lambda b2c which contains a deletion is the least dense of the three phages tested and is inactivated the least. Lambda c1857am S7 is intermediate in density and was inactivated the most while lambda d*met* 145, most dense, inactivated most rapidly. Using the established inactivation and transduction procedures we have isolated several lysogens of presumptive lambda d*met* 145 deletion mutants which carry either the *met* B and *met* F genes or the *met* F gene.

DISCUSSION

Although the original procedure for studying methionine regulation was abandoned, the second experimental technique may yield the same result in that the presumptive lamgda d*met* 145 deletion mutants may be used to identify the promotors of the methionine regulon. A promotor is the site which signals the DNA dependant RNA polymerase to begin transcription to form messenger RNA. This site is requisite for methionine biosynthesis. The original objective of producing gene fusion products is still possible in that the methionine genes of some lambda d*met* 145 deletion mutants may be fused to the structural promotors on the viral portion of the transducing phage chromosome. In addition, deletion of certain elements of the *met* BJLMF cluster may reveal the position of controlling elements for the four genes.

In conclusion, our major accomplishment this semester

has been the establishment of a reliable procedure for the selection of desired deletion mutants and the isolation of presumptive lambda d*met* 145 deletion mutants which may be used in the study of methionine regulation in *Escherichia coli*.

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