JOURNAL OF BACTERIOLOGY, Aug. 1980, p. 726-730
0021-9193/80/08-0726/05$02.00/0

Vol. 143, No. 2

Regulation of Fatty Acid Degradation in *Escherichia coli*: Dominance Studies with Strains Merodiploid in Gene *fadR*

ROBERT W. SIMONS,† KELLY T. HUGHES, AND WILLIAM D. NUNN*

Department of Molecular Biology and Biochemistry, University of California at Irvine, Irvine, California 92717

Strains stably merodiploid in the 25-min region of the chromosome of *Escherichia coli* were constructed and used in dominance tests between various wild-type and mutant alleles of the *fadR* gene. Whereas the monoploid *fadR*$^+$ and *fadR* strains were inducible and constitutive, respectively, for the enzymes involved in fatty acid degradation (*fad*), merodiploids with at least one *fadR*$^+$ allele were inducible. This observation was true whether the *fadR*$^+$ allele resided on the main chromosome or on the episome. These results show that *fadR*$^+$ is *trans* dominant to *fadR*, and they are consistent with the proposal that the *fadR* gene product is a repressor protein. Complementation tests were also performed by constructing 24 merodiploids harboring *fadR* alleles on both the main chromosome and the episome. All of these *fadR*/*fadR* diploids were able to utilize the noninducing substrate decanoate as sole carbon source, suggesting that only one polypeptide is encoded by the *fadR* gene.

The enteric bacterium *Escherichia coli* oxidizes fatty acids by cyclic β-oxidation and thiolytic cleavage (8). Through the isolation and characterization of mutants unable to utilize fatty acids, Overath and his co-workers were able to show that the structural genes for the enzymes catalyzing fatty acid degradation (*fad*) map at no fewer than three separate locations on the *E. coli* chromosome (3, 9). When the wild type is grown in the presence of long-chain (>C_{12}) fatty acids, the activities of the five key β-oxidative enzymes are coordinately induced (3, 8, 14). In contrast, although the medium chain (C_6 to C_{11}) fatty acids can serve as substrates for these *fad* enzymes, they cannot themselves induce the *fad* enzymes (8, 14). Mutants able to utilize medium-chain fatty acids as sole carbon sources are readily selected by plating the wild type onto decanoate (8, 14). Overath and co-workers first showed that mutants obtained in this way were constitutive for the *fad* enzymes and could now rapidly oxidize both medium- and long-chain fatty acids (8). These mutants have been termed *fadR* (10), and Overath et al. (8) have suggested that the gene codes for a regulatory protein, possibly a repressor.

Several lines of evidence now support Overath's original contention, indicating that the *fadR* gene product is a diffusible repressor protein. First, data quoted by Vanderwinkel et al. (12) and more recent results by Simons et al. (11) show that the *fadR* gene maps at a locus distinct from all other *fad* loci, suggesting that the product is diffusible, acting in *trans* to control *fad* gene expression. Second, mutants that harbor Tn*10* insertions in the *fadR* gene have been isolated and shown to be constitutive for the *fad* enzymes (11). In view of the observation that Tn*10* insertion totally disrupts gene activity (2), this result suggests that the *fadR* gene product is a repressor. Third, the recent characterization of *fadR*(Ts) mutants (11), which are inducible at low temperatures but constitutive at elevated temperatures, lends additional support to the repressor model.

In this communication, we report studies with strains that are stably merodiploid in the *fadR* gene. Our results show that *fadR*$^+$ is *trans* dominant to *fadR*, whether situated on the episome or the chromosome, confirming that the *fadR* gene product is a repressor, operating negatively to control the *fad* regulon. Furthermore, complementation studies indicate that the *fadR* gene codes for only one polypeptide.

MATERIALS AND METHODS

Bacterial strains used. All bacterial strains used in this study were derivatives of K-12 and are listed in Table 1. Some of the merodiploid strains constructed for dominance tests are listed in Table 2. Mutagenesis of the *fadR* gene with Tn*5* (transposon encoding resistance to kanamycin, Kn^r) (1) was accomplished by infection of JC268 with λ*rex*::Tn*5*cI857 followed by simultaneous selection for Kn^r and growth on decanoate (Dec$^+$). This procedure was similar to that described for the Tn (transposon encoding tetracycline

† Present address: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

TABLE 1. *Bacterial strains used*

Strain	Relevant genotype[a]	Source or reference
H680	F⁻ *purB51 trp-45 his-68 tyrA2*	P. deHaan strain via CGSC[b]
JK268	F⁻ *purB58 trpA62 trpE61*	J. Kuhn strain via CGSC
K12	F⁺ prototrophic	J. Lederberg strain via CGSC
KL166	Hfr *gyrA13*	
KLF25/KL181	F125/*thi-1 pyrD34 his-68 trp-45 recA1 rpsL118*	B. Low strain (4)
KLF26/KL181	F126/*thi-1 pyrD34 his-68 trp-45 recA1 rpsL118*	B. Low strain (4)
NK5304	Hfr *srlA*::Tn*10 recA thr-300 ilv-318*	N. Kleckner strain
RS1026	F⁻ *purB51 trp-45 tyrA2*	R. Simons et al. (11)
RS1073	F⁻ *purB51 trp-45 his-68*	R. Simons et al. (11)
RS3039	Hfr *metB1 purB58 fadR13*::Tn*10 hemA42*	R. Simons et al. (11)
RS3069	F⁻ *purB58 fadR17*::Tn*5 trpA62 trpE61*	R. Simons et al. (11)
RW208	F⁻ H680 *his*⁺ *gyrA13 fadR17*::Tn*5 srlA*::Tn*10 recA*	R. Simons et al. (11)
RW223	F⁻ RS1026 *nalA recA srlA*::Tn*10*(Tc[s])	R. Simons et al. (11)
RW226-1	F⁻ RW223 *fadR23*	R. Simons et al. (11)
RW227	F⁻ RS1073 *fadR17*::Tn*5 srlA*::Tn*10 recA*	R. Simons et al. (11)
RW229	F⁻ H680 *tyr*⁺ *srlA*::Tn*10 recA*	This work
RW231-1	F⁻ RW229 *fadR19*	This work
RW231-2	F⁻ RW229 *fadR20*	This work
RW231-3	F⁻ RW229 *fadR21*	This work
RW231-4	F⁻ RW229 *fadR22*	This work

[a] Only relevant or auxotrophic markers are listed. See references for complete genotypes. Allele numbers of *fadR* mutations are ours; all others are those of the source.

[b] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

resistance, Tc[r] [2]) mutagenesis of *fadR* (11). Strain RS3069 was obtained in this mutagenesis and was found by genetic and biochemical characterization (data not shown) to bear a *fadR*::Tn*5* mutation.

Strains RS1026 and RS1073 were *his*⁺ and *tyr*⁺ derivatives, respectively, of strain H680, obtained after transduction with P1 *vir* grown on K-12. Strains harboring the *nalA* mutation were constructed by transduction with P1 *vir* grown on KL166, followed by selection for resistance to nalidixic acid (50 μg/ml) as described by Miller (6). The *fadR*::Tn*5* derivatives were obtained by selecting for Kn[r] (30 μg/ml) after infection with P1 *vir* grown on RS3069. To construct derivatives defective in homologous recombination (*recA*), cells were infected with P1 *vir* grown on NK5304, followed by selection for resistance to tetracycline (20 μg/ml). The *srlA*::Tn*10* and *recA* markers are about 50% cotransducible, and the desired *recA* cotransductants were identified by their sensitivity to nitrofurantoin (2 μg/ml) (5). In some cases, *srlA*::Tn*10 recA*⁺ (nitrofurantoin-resistant) transductants were isolated in similar transductions.

Bochner et al. (B. R. Bochner, H. Huang, G. L. Schieven, and B. N. Ames, submitted for publication) recently developed a method for the direct selection of tetracycline-sensitive (Tc[s]) derivatives of strains harboring Tn*10*. This approach is based upon the sensitivity of Tn*10*-bearing strains to quinaldic and fusaric acids. We used this technique for the selection of Tc[s] derivatives of *srlA*::Tn*10* strains (strains RW223, W3 Tc[s], and W48 Tc[s]). These strains were Srl⁻ and did not revert to Tc[r] (<1 revertant per 10^7 bacteria); however, no attempt was made to determine whether any portion of the Tn*10* element remained.

The *fadR* strains RW231-1, RW231-2, RW231-3, and RW231-4 were selected from four separate clones of RW229 for growth on decanoate, thus minimizing the chance that these strains were the result of identical mutational events. Similarly, strains D1, D2, D3, D4, D5, and D6 were selected from independent clones of the merodiploid strain W3 Tc[s] on decanoate. These latter strains were shown to harbor mutations in the episomal *fadR* gene (see Results). RW226-1 was a *fadR* derivative of RW223.

Media and culture conditions. The minimal medium used was medium E (13). Rich broth and Luria broth medium were prepared according to Miller (6). Fatty acids were provided at 5 mM in the presence of Brij 58 (5 mg/ml); D-glucose, at 25 mM; and potassium acetate, at 50 mM. Amino acids were supplemented at 0.01%; adenine sulfate, at 0.004%; and δ-aminolevulinic acid, at 0.04%. Tetracycline was used at 20 μg/ml; kanamycin, at 30 μg/ml; nalidixic acid, at 50 μg/ml; and nitrofurantoin, at 2 μg/ml. Plates used in the selection of Tc[s] derivatives of Tn*10*-bearing strains were according to Bochner et al. (submitted). For faster growing strains, lowering the D-glucose concentration in these plates to 5.5 mM improved Tc[s] selection (we are indebted to S. Maloy for this suggestion). All cells were routinely grown at 37°C in a New Brunswick gyratory shaking water bath.

Genetic techniques. The bacteriophage P1 *vir* was used in all transductions. Preparation of phage lysates and transductions were as described (7). Matings were done essentially according to Miller (6), at 37°C for 2 h.

Segregation and curing of episomes. Most of the merodiploids constructed in Table 2 were somewhat unstable when grown in rich broth, giving rise to ca. 5 to 20% monoploid segregants after growth overnight. These merodiploids gave rise to less than 0.3% monoploid segregants after growth overnight in minimal medium lacking adenine and tryptophan. When it was difficult to obtain spontaneous monoploid segregants, merodiploids were cured of their episome with acridine orange by the method of Miller (6).

Biochemical procedures. Measurement of the β-oxidation of [1-^{14}C]oleate was performed with whole cells as described (7). Specific activities of the individual β-oxidation enzymes were determined spectrophotometrically from French press extracts of whole cells, as described (7). Protein concentrations were determined by a microbiuret procedure (7).

Materials. Most reagents used in this study were as described (11). Nitrofurantoin, quinaldic acid, fusaric acid, and chlortetracycline were purchased from Sigma Chemical Co., St. Louis, Mo.

TABLE 2. *Construction of merodiploid strains used in dominance tests*

Strain	Donor[a]	Recipient[b]	Selected markers	Relevant genotype of merodiploid[c]	Diploidy[d] of fadR	Phenotype[e]
M11	W3 Tc^s	RW229	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125/*purB trp*	+/+	FadR$^+$
M62	W48 Tc^s	RW229	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F126/*purB trp*	+/+	FadR$^+$
M2	W3 Tc^s	RW231-2	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125/*purB fadR20 trp*	+/−	FadR$^+$
M4	W48 Tc^s	RW231-2	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F126/*purB fadR20 trp*	+/−	FadR$^+$
M47	D1	RW229	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125 *fadR23/purB trp*	−/+	FadR$^+$
M48	D2	RW229	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125 *fadR24/purB trp*	−/+	FadR$^+$
M23	D1	RW231-1	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125 *fadR23/purB fadR19 trp*	−/−	FadR$^-$
M30	D2	RW231-4	PurB$^+$ Trp$^+$ *tyr*$^+$ Tc^r	F125 *fadR24/purB fadR22 trp*	−/−	FadR$^-$
W3	KLF25/KL181	RW208	PurB$^+$ Trp$^+$ *his*$^+$ *nalA*	F125/*purB fadR17*::Tn*5 trp*	+/Tn*5*	FadR$^+$
W48	KLF26/KL181	RW208	PurB$^+$ Trp$^+$ *his*$^+$ *nalA*	F126/*purB fadR17*::Tn*5 trp*	+/Tn*5*	FadR$^+$
M63	D1	RW227	PurB$^+$ Trp$^+$ *his*$^+$ *nalA*	F125 *fadR1/purB fadR17*::Tn*5 trp*	−/Tn*5*	FadR$^-$

[a] All donors were *recA*. Strains W3 Tc^s and W48 Tc^s were tetracycline-sensitive derivatives of strains W3 and W48, respectively. Strains D1 and D2 were independent, spontaneous Dec$^+$ derivatives of W3 Tc^s.

[b] The construction of these strains is detailed in the text. Strains RW231-1, RW231-2, and RW231-4 were independent, spontaneous Dec$^+$ derivatives of strain RW229.

[c] Only relevant genotype shown. See Table 1 for complete genotype. All merodiploids were *recA*.

[d] Nomenclature of alleles: +, *fadR*$^+$; −, *fadR*; Tn*5*, *fadR*::Tn*5*. Notation to left of slash refers to episomal allele; that to right of slash refers to chromosomal allele.

[e] Phenotypes: FadR$^+$, unable to utilize decanoate (Dec$^-$); FadR$^-$, able to utilize decanoate (Dec$^+$).

RESULTS

Construction of strains merodiploid in gene *fadR*. The *fadR* gene has previously been shown to map at ca. 25.5 min on the *E. coli* chromosome (11) and is flanked by the genetic markers *purB, hemA*, and *trp* (Fig. 1). To examine dominance between various alleles of *fadR*, we constructed a series of strains merodiploid for the genes in this 25-min region. Several available F-primes reportedly carry chromosomal genes in this region (4). Two of these episomes, F125 and F126 (Fig. 1), previously isolated and characterized by Low (4), were used in the dominance studies reported here. Both F125 and F126 were derived from Hfr Broda 7 and carry genes complementing markers that flank the *fadR* gene (4). When we examined Hfr Broda 7, we found it to be *fadR*$^+$ (data not shown). It therefore seemed reasonable that the episomes F125 and F126 carried the *fadR*$^+$ gene as well. In the course of this work, F125 was found to be a more stable F-prime than F126, and most strains were therefore constructed with this episome.

The manner of construction and relevant genotypes of merodiploids used in these dominance tests are described in Table 2. In all strains, the host chromosome harbored auxotrophic mutations in the *purB* and *trp* genes (see Table 1) which were complemented by the respective wild-type alleles on the F-prime. This arrangement not only facilitated strain construction but also prevented segregation of the episome when

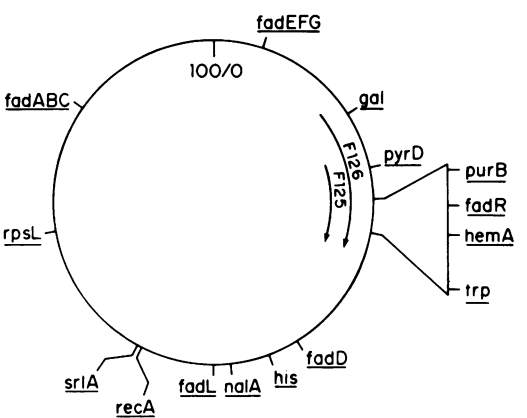

FIG. 1. *Chromosome of E. coli with relevant markers and the episomes F125 and F126. The fadR region is enlarged to indicate relative positions of markers.*

the merodiploids were grown in the absence of adenine and tryptophan. The host strains were all *recA* derivatives of strain H680 and differed only in certain markers that proved useful in counterselection after matings. The isolation of F-primes that carried a mutation in the *fadR* gene is described in Materials and Methods.

Examination of the growth properties of the merodiploids in Table 2 suggested that the *fadR*$^+$ allele was *trans* dominant to *fadR*. All merodiploids, in which at least one of the *fadR* alleles was wild type, were phenotypically FadR$^+$ (Dec$^-$). This was true whether the dom-

inant allele resided on the episome or on the main chromosome.

Proof of merodiploidy and isolation of segregants. The merodiploid strains in Table 2 were all *recA* and therefore defective in homologous recombination between the F-prime and the main chromosome. The genes present on the episome were thus genetically distinct from those of the main chromosome. To prove the diploidy of the *fadR* gene in these stable merodiploids, we examined linkage relationships of episomal and chromosomal genes in several ways. First, monoploid derivatives of each merodiploid were obtained either by spontaneous segregation of the episome during growth in rich broth or by curing with acridine orange. These segregants were recognized by the auxotrophic requirements for adenine and tryptophan, as well as an inability to transfer markers in further matings. All segregants were examined for growth on decanoate and found to be phenotypically the same as the original monoploid host strain. Second, each merodiploid in Table 2 was examined for the ability to transfer the *purB*$^+$ and *trp*$^+$ markers in matings with appropriate *recA* recipients (RW223, RW226-1), and all were found to be fertile (data not shown). In matings involving transfer of an episome thought to bear a *fadR*$^+$ allele to an appropriate *fadR* (Dec$^+$) strain (RW226-1), the F-ductants were always found to be phenotypically FadR$^+$ (Dec$^-$). Third, P1 transductions confirmed the linkage between *fadR* alleles and the appropriate episomal or chromosomal markers (data not shown). It was concluded that the strains under study (Table 2) are indeed diploid for the *fadR* gene and that the episomal and chromosomal *fadR* alleles are correct as stated.

Beta-oxidation in strains monoploid and diploid for various *fadR* alleles. In wild-type *E. coli*, the ability to rapidly oxidize fatty acids is induced only during growth in the presence of long-chain fatty acids (e.g., oleate, C_{18}). In contrast, *fadR* mutants are constitutive for this oxidation, requiring no induction. It is this constitutivity that allows growth of *fadR* mutants on the noninducing medium-chain fatty acids (e.g., decanoate) (8, 14). The growth behavior of merodiploids containing both a mutant and a wild-type allele of the *fadR* gene suggested that they were phenotypically FadR$^+$ (Table 2). To determine dominance, several monoploid and merodiploid strains were grown in minimal medium under both inducing and noninducing conditions. Cells from these cultures were then examined for their ability to oxidize [1-^{14}C]oleate. As long as one of the *fadR* alleles in these merodiploids was *fadR*$^+$, these strains were inducible for oleate oxidation (data not shown). Merodiploids mutant for both episomal and chromosomal *fadR* alleles were constitutive.

These observations were confirmed by determination of the specific activities of the five key β-oxidative enzymes in extracts of various monoploid and merodiploid strains grown under both inducing and noninducing conditions. The results are presented in Table 3 and clearly show that *fadR*$^+$/*fadR* merodiploids are inducible for these enzyme activities. These results demonstrate that *fadR*$^+$ is dominant to *fadR*.

Complementation analysis between various *fadR* alleles. Six merodiploids (D1, D2, D3, D4, D5, and D6) carrying independent, spontaneous *fadR* mutations on their episome and four independent haploid strains (RW231-1, RW231-2, RW231-3, and RW231-4) each carrying a spontaneous *fadR* mutation on the chromosome were mated in a pairwise fashion. The resulting 24 merodiploids were examined by transductional analysis of their episomal and chromosomal linkage groups, and all were found to be *fadR*/*fadR* (data not shown). All 24 merodiploids were Dec$^+$ and therefore phenotypically FadR$^-$. These results indicate that all of

TABLE 3. *Specific activities of β-oxidative enzymes from extracts of monoploid and merodiploid strains*[a]

Strain	Ploidy of *fadR*	Growth carbon source	Oleoyl-CoA synthetase	Palmitoyl-CoA dehydrogenase	Crotonase	β-Hydroxybutyryl-CoA dehydrogenase	Acetoacetyl-CoA thiolase
M11	+/+	Acetate	0.30	0.32	1.67	4.0	1.2
		Acetate + oleate	1.58	2.05	677	483	40
M2	+/−	Acetate	0.24	0.16	2.99	2.0	1.1
		Acetate + oleate	1.78	2.14	655	467	32
M47	−/+	Acetate	0.28	0.32	4.46	7.0	1.9
		Acetate + oleate	2.36	3.04	886	452	35
M23	−/−	Acetate	1.94	3.01	680	500	34
		Acetate + oleate	1.93	2.68	472	605	36

[a] CoA, Coenzyme A. Enzyme activities are expressed as nanomoles per minute per milligram of protein.

the *fadR* mutations examined reside in a single complementation group.

DISCUSSION

The dominance studies reported here demonstrate that *fadR*$^+$ is *trans* dominant to the constitutive *fadR* allele. A simple explanation for these results is that the product of the *fadR* gene is a negatively controlling element or repressor. If the *fadR* gene product controlled the *fad* regulon in a simple positive fashion, then one would expect the constitutive *fadR* mutation to be dominant to *fadR*$^+$, contrary to our observations.

The *fadR* mutants used in these experiments were obtained during selection for the ability to utilize decanoate as a sole carbon source. All *fadR* mutants that have been described to date were obtained in similar selection schemes (8, 14). A number of such spontaneous *fadR* mutants were recently examined genetically, and all were found to map at the same locus (11). Mutations in *fadR* that render the cell noninducible for the *fad* enzymes have not yet been described. If the *fadR* gene product is a repressor, such noninducible *fadR* alleles, analogous to the *lacI*^{s} super-repressor (15), would be predicted to be dominant to both *fadR*$^+$ and the constitutive *fadR* alleles.

In the complementation experiments reported here, we examined a total of 10 independent *fadR* mutations in various pairwise arrangements. Our criterion for complementation between any two *fadR* mutations was loss of the ability of the merodiploid to utilize decanoate as a sole carbon source. All 24 of the *fadR*/*fadR* merodiploids examined remained able to grow on decanoate, and on the basis of these experiments, we conclude that only one polypeptide is encoded by the *fadR* locus. It is possible, however, that a more extensive complementation analysis may reveal additional polypeptides encoded at this locus.

In summary, all available evidence regarding regulation of *fad* gene expression in *E. coli* is consistent with Overath's original proposal (8) that the product of the *fadR* gene is a diffusible repressor protein: (i) constitutive *fadR* mutations occur at a relatively high spontaneous frequency (8, 11, 14); (ii) insertion mutations in *fadR* result in constitutivity (11); (iii) *fadR*(Ts) mutants are thermally inducible (11); and (iv), as reported here, *fadR*$^+$ is dominant to *fadR*.

ACKNOWLEDGMENTS

We are grateful to Barbara Bachmann for the gift of numerous strains and to Paula Hennen and Stanley Maloy for critically reading the manuscript.

This work was supported by Public Health Service grant GM22466-1A from the National Institute of General Medicine. W.D.N. is an established investigator of the American Heart Association. R.W.S. was supported by Public Health Service training grant GM07311 from the National Institute of General Medicine.

LITERATURE CITED

1. **Berg, D. E.** 1977. Insertion and excision of the transposable kanamycin resistance determinant Tn*5*, p. 205–212. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Botstein, D., and N. Kleckner.** 1977. Translocation and illegitimate recombination by the tetracycline resistance element Tn*10*, p. 185–203. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Klein, K., R. Steinberg, B. Fiethen, and P. Overath.** 1971. Fatty acid degradation in *Escherichia coli*. An inducible system for the uptake of fatty acids and further characterization of old mutants. Eur. J. Biochem. **19**:442–450.
4. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
5. **McEntee, K.** 1977. Genetic analysis of the *Escherichia coli* K-12 *srl* region. J. Bacteriol. **132**:904–911.
6. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Nunn, W. D., R. W. Simons, P. A. Egan, and S. R. Maloy.** 1979. Kinetics of the utilization of medium and long chain fatty acids by a mutant of *Escherichia coli* defective in the *fadL* gene. J. Biol. Chem. **254**:9130–9134.
8. **Overath, P., G. Pauli, and H. U. Schairer.** 1969. Fatty acid degradation in *Escherichia coli*. An inducible acyl-CoA synthetase, the mapping of *old*-mutations, and the isolation of regulatory mutants. Eur. J. Biochem. **7**: 559–574.
9. **Overath, P., E. Raufuss, W. Stoffel, and W. Ecker.** 1967. The induction of the enzymes of fatty acid degradation in *Escherichia coli*. Biochem. Biophys. Res. Commun. **29**:28–33.
10. **Pauli, G., and P. Overath.** 1972. *ato* operon: a highly inducible system for acetoacetate and butyrate degradation in *Escherichia coli*. Eur. J. Biochem. **29**:553–562.
11. **Simons, R. W., P. A. Egan, H. T. Chute, and W. D. Nunn.** 1980. Regulation of fatty acid degradation in *Escherichia coli*: isolation and characterization of strains bearing insertion and temperature-sensitive mutations in gene *fadR*. J. Bacteriol. **142**:621–632.
12. **Vanderwinkel, E., M. De Vlieghere, M. Fontaine, D. Charles, F. Denamur, D. Vandevoorde, and D. De Kegel.** 1976. Septation deficiency and phospholipid perturbation in *Escherichia coli* genetically constitutive for the beta oxidation pathway. J. Bacteriol. **127**: 1389–1399.
13. **Vogel, H. J., and D. M. Bonner.** 1956. Acetyl-ornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
14. **Weeks, G., M. Shapiro, R. O. Burns, and S. J. Wakil.** 1969. Control of fatty acid metabolism. I. Induction of the enzymes of fatty acid oxidation in *Escherichia coli*. J. Bacteriol. **97**:827–836.
15. **Willson, C., D. Perrin, M. Cohn, F. Jacob, and J. Monod.** 1964. Non-inducible mutants of the regulator gene in the lactose system of *Escherichia coli*. J. Mol. Biol. **8**:582–592.