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Genetic studies of D-alanine-dehydrogenase-less mutants of *Escherichia coli* K12

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SUMMARY

Genetic analysis of 12 mutants of *Escherichia coli* K12 defective in D-alanine dehydrogenase showed that *alnA* and *dad* are alternative names for the same locus. *dad* was shown to be a single gene which codes for a protein of 55000–60000 mol. wt. Study of thermosensitive mutants of *dad* indicated that its product is a structural component of D-alanine dehydrogenase. The regulatory gene *alnR* was shown to be involved in positive control of *dad* expression. This was concluded from (i) the absence of constitutive strains among Dad$^+$ revertants of *alnR*$^-$ mutations, (ii) the *trans* dominance of *alnR*$^+$ to *alnR*$^-$, and (iii) the failure to isolate fully constitutive strains by any means. To obtain a uniform nomenclature it is proposed to re-name *dad* as *dadB* and *alnR* as *dadQ*.

1. INTRODUCTION

Escherichia coli can utilize D-alanine as sole source of carbon and nitrogen for growth. Mutants which have lost this capacity (Dad$^-$) were described by Beelen, Feldmann & Wijsman (1973) and by Franklin & Venables (1976). In both laboratories the mutations producing the Dad$^-$ phenotype were mapped between *ara* and *leu* on the *E. coli* linkage map. Beelen *et al.* (1973) described the biochemical lesion as a loss of the enzyme 'alaninase' which deaminates D-alanine to pyruvate, and named the locus *alnA*. Franklin & Venables (1976) described the lesion in their mutants as the loss of a membrane-bound, respiratory chain linked, D-alanine dehydrogenase, and named the locus *dad*. It seemed very likely that *alnA* and *dad* were alternative names for the same locus which coded for an oxidative deaminase, specific for D-amino acids and essential for the utilization of D-alanine by *E. coli*.

Another Dad$^-$ mutant isolated by Beelen *et al.* (1973), GIA68, mapped on the

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other side of *thr* from *alnA* at about minute 99 on the current linkage map. In a temperature sensitive revertant of this mutant, induction of D-alanine dehydrogenase synthesis was defective at the non-permissive temperature whereas the preformed enzyme activity was temperature insensitive. This indicated that GIA68 was defective in a regulatory gene, possibly involved in positive control of D-alanine dehydrogenase induction. This gene was named *alnR*.

In the present paper the relationships between the *dad* mutants of Franklin & Venables (1976) and the *alnA* and *alnR* mutants of Beelen *et al.* (1973) are further investigated, and the following conclusions are reached: *alnA* and *dad* are genetically identical; *dad* is a single gene which codes for a structural component of D-alanine dehydrogenase; expression of *dad* is regulated by *alnR* in a positive or positive–negative control system. To facilitate explanation of results and reference to previously published work, the genetic symbols *dad* and *alnA* are both used in this paper. However, they should be regarded as synonymous, and a rationalized nomenclature which takes other related genes into consideration is proposed in the Discussion section.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

The bacterial strains used are listed in Table 1. All *aln* or *dad* mutants are defective in the enzyme D-alanine dehydrogenase and fail to grow when alanine isomers are supplied as sole carbon source (Dad$^-$ phenotype). CF2, 3, 4, 10, 11, 13 & 201 were previously described by Franklin & Venables (1976).

(ii) *Media, culture methods etc.*

Growth media, culture methods, cell breakage and fractionation, and preparation of membranes were as described previously (Franklin & Venables, 1976).

(iii) *Induction of enzyme activity*

Except for Table 5 (where cells were grown on alanine) induction of D-alanine dehydrogenase was accomplished by growing cells to late exponential phase in succinate minimal medium, washing in 50 mM sodium/potassium phosphate buffer (pH 7·2) and incubating in the same buffer plus alanine (33 mM).

(iv) D-*alanine dehydrogenase assay*

The enzyme was measured by following the reduction of 2,6-dichlorophenol-indophenol (DCPIP) spectrophotometrically in a phenazine methosulphate linked assay as described by Franklin & Venables (1976). All assays were carried out on the particulate cell wall/membrane fraction. Protein was estimated by the method

of Lowry *et al.* (1951) and D-alanine dehydrogenase specific activity is expressed as n moles DCPIP reduced/min/mg protein.

(v) *Mutant isolation*

Mutants defective in D-alanine dehydrogenase were selected from AB259 by their inability to grow of DL-alanine following treatment with *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine (MNNG), ampicillin selection and replica plating. Thermosensitive revertants of *dad* mutants were selected by plating about 2×10^8

Table 1. *Bacterial strains*

Strain	Relevant markers and other information
AB259	HfrH *thi*
W945	*thr ara leu thi*
GIA39	*thr leu pyrF his arg ilvA thi alnA* (Beelen *et al.* 1973)
KMBL146	*thr leu pyrF thy his arg ilvA thi* (Beelen *et al.* 1973)
GIA68	KMBL146 *alnR* (Beelen *et al.* 1973)
KL16-99	Hfr *recA*
CF1, 2, 4, 10, 11 25, 105, 106	*dad* point mutations of AB259
CF3, 13, 201	*dad* deletions of AB259
CF2ts4	thermosensitive Dad^+ revertant of CF2
CF10ts3	thermosensitive Dad^+ revertant of CF10
CF240	GIA68 *recA*/F thr^+ leu^+ $alnR^+$
CF251–255	Five Dad^+ revertants of GIA68

MNNG-treated cells to DL-alanine and incubating for 4 days at 28 °C. Colonies which formed were re-streaked to DL-alanine plates and those which showed good growth at 28 °C coupled with weak growth at 40 °C were retained. Revertants of the *alnR* mutant GIA68 were selected by the same method but with all incubations at 37 °C.

(vi) *Genetic exchange*

Transduction was carried out with phage *Plkc* by the methods of Lennox (1955). In crosses where both donor and recipient carried mutant *dad* alleles, multiplicity of infection was 10. In other crosses multiplicity of infection was 2. Conjugation was carried out by growing donor and recipient cultures to mid-exponential phase in Nutrient Broth ($E_{550} = 4$–6), mixing in the ratio 1 donor : 10 recipient and continuing incubation for up to 2 h. Recombinants were then selected on appropriate media.

(vii) *Electrophoresis*

The methods of Churchwood & Holland (1976) were followed. Membrane preparations were solubilized by heating at 100 °C for 5 min, in a mixture containing sodium dodecyl sulphate (SDS) (1%), glycerol (10%), dithiothreitol (1%) and bromophenol blue (0·002%). Protein concentration was adjusted to 5 mg/ml and 2–4 ul. aliquots were run on 12·5% acrylamide/SDS gels in Tris/glycine buffer (pH 7·3). Gels were stained in Coomassie brilliant blue.

3. RESULTS

(i) *Fine-structure analysis of the* dad *locus*

P1-mediated transductional crosses were carried out amongst a selection of 5 *dad* mutants previously described by Franklin & Venables (1976), 7 newly-isolated *dad* mutants and GIA39, the *alnA* mutant of Beelen *et al.* (1973). Initial crosses were between the 3 strains carrying *dad* deletions CF3, CF13 (a leu^- derivative), and CF201, selecting leu^+ and dad^+ transductants. Leu^+ transductants were obtained at high frequency (12–42/10^7 *P1*) in all crosses, whereas dad^+ transductants were obtained at low frequencies (0·25–0·4/10^7 *P1*) in CF3 × CF13 and CF3 × CF201 crosses and not at all in CF13 × CF201 crosses. These results, together with previously obtained data on the extents of the deletions involved (*dad-3* extends into *ara*; *dad-201* extends into *leu*, *dad-13* is not known to extend into other markers; Franklin & Venables, 1976) were used to compile the deletion map shown in Figure 1. The lack of detectable recombination between *dad-13* and *dad-201* indicates either that the two deletions overlap or that their termini are so close that recombination cannot be detected by the method used. Further results favour the former interpretation.

The remaining nine mutants (CF1, 2, 4, 10, 11, 25, 105, 106 and GIA39) which are point mutants showing MNNG-induced reversion were crossed with each of the 3 deletion mutants as shown in Table 2. Selected markers were dad^+ and either ara^+ or leu^+ as transduction controls. In each cross the occurrence of dad^+ transductants was taken to indicate that the point mutation concerned was located outside the region of deletion against which it was crossed, whereas their absence was taken to indicate that the point mutation was located within the region of the deletion. In this way the mutants were divided into 4 groups (*dad-1* is the allele carried by CF1 *etc*): *dad-11* was overlapped only by the *dad-3* deletion; the *alnA* mutation (GIA39) and *dad-105* only by the *dad-13* deletion; *dad-1* and *dad-10* by both the *dad-13* and *dad-201* deletions; *dad-2*, *-4*, *-25*, and *-106* only by the *dad-201* deletion (Figure 1). The order of the point mutations within each of these groups was determined by the following nineteen *P1*-mediated crosses between selected pairs of alleles (donors first): all *dad* point mutations including *alnA1* × *dad-106* and × *dad-11*; *dad-105* × *alnA1*; *dad-1* × *dad-10*; and *dad-2* × *dad-4*. Results from 2 representative crosses are shown in Table 3. Drastic reduction of apparent

linkage to *ara* amongst transductants selected as *dad*$^+$ (CF1 × W945 *dad-106*) is assumed to be indicative of a requirement for quadruple exchanges in the generation of *dad*$^+$ *ara*$^+$ recombinants and, therefore, that the donor *dad* allele lies between the recipient *dad* allele and *ara*. Conversely, where the apparent *dad-ara* linkage (CF4 × W945 *dad-106*) is comparable to the *dad-ara* cotransduction

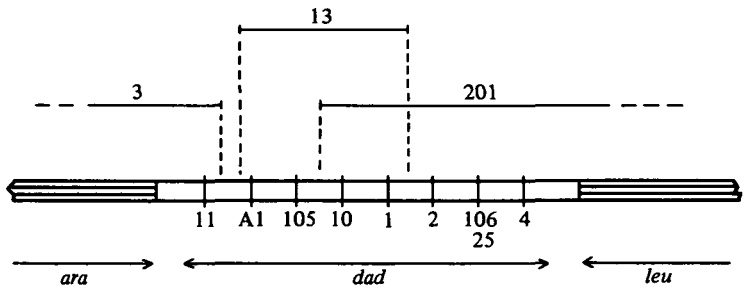

Fig. 1. Fine structure of the *dad* locus. All numbers refer to *dad* allele numbers as described in the text. *A1* is the *alnA1* allele of Beelen *et al.* (1973). Approximate extents of deletions *3*, *13* and *201* are shown. *106* and *25* are assumed to be the same allele as recombination between them could not be detected.

frequency obtained with a wild-type donor (AB259 × W945 *dad-106*), it is assumed that *dad*$^+$ *ara*$^+$ arises by double exchanges and therefore that the donor *dad* allele does not lie between the recipient *dad* and *ara*. The same rationale was used in the interpretation of all crosses; the minimum apparent *dad-ara* cotransduction frequency interpreted to indicate double exchanges was 58% and the maximum interpreted to indicate quadruple exchanges was 12%; no intermediate values were obtained. All data were consistent with the allele order shown in Figure 1.

In *P1*-mediated crosses between wild-type donor and *dad* mutants abortive transductants can normally be detected at a frequency of about 8 abortive transductants: 1 complete transductant. In the above crosses where both donor and recipient carried *dad* mutations abortive transductants were never detected. This is evidence for lack of complementation between *dad* mutations and indicates that, collectively, they represent a single gene. The fact that the *alnA1* allele of Beelen *et al.* (1973) maps amongst the *dad* mutations and complements none of them, proves that *dad* and *alnA* are the same gene.

(ii) *The product of* dad

Levels of D-alanine dehydrogenase are much reduced in all *dad* mutants and in deletions they are undetectable (Beelen *et al.* 1973; Franklin & Venables, 1976). Beelen and colleagues assumed that *dad* was the structural gene for this enzyme but direct evidence was lacking. To clarify the function of *dad* we isolated thermosensitive revertants of the mutants CF2 and CF10. These mutants, CF2ts4

and CF10ts3 showed moderate to good growth on alanine plates at 28 °C but no growth at 40–42 °C. The results shown in Table 4 clearly indicate that in these revertants it is the dehydrogenase itself which is thermosensitive and not the induction process. From the data in Table 4 it might appear that induction of the enzyme synthesis is defective in CF10ts3 at 40 °C but in this mutant thermal inactivation of the dehydrogenase is rapid and irreversible, with the result that

Table 2. *Recombination between* dad *point mutations and* dad *deletions CF3, CF13 and CF201*

Donor strains	Occurrence of dad$^+$ recombinants in crosses with recipients		
	CF13 *leu*$^-$	CF3	CF201
CF1	−	+	−
CF2	+	+	−
CF4	+	+	−
CF10	−	+	−
CF11	+	−	+
CF25	+	+	−
CF105	−	+	+
CF106	+	+	−
GIA39 *leu*$^+$	−	+	+

A *leu*$^-$ mutation was introduced into CF13 by nitrosoguanidine mutagenesis. GIA39 was made *leu*$^+$ by transduction with AB259 as donor. + donotes occurrence of *dad*$^+$ transductants at frequencies of 12–230/10^9 *P1*. Use of *dad* deletions as recipients eliminated interference from revertants to *dad*$^+$. In all crosses *leu*$^+$ transductants (recipients CF13 and CF201) or *ara*$^+$ transductants (recipient CF3) occurred at frequencies of 50–252/10^7 *P1*.

Table 3. *Examples of crosses used to determine the map order of* dad *alleles*

Donor	Recipient	Selected marker	% scored as *ara*$^+$
AB259	W945 *dad-106*	*dad*$^+$	82
CF1	W945 *dad-106*	*dad*$^+$	5
CF4	W945 *dad-106*	*dad*$^+$	68

W945 *dad-106* was obtained as an *ara*$^-$ *dad*$^-$ *leu*$^+$ transductant from a C106 × W945 cross. Genotypes of donors are: AB259, + + +; CF1, + *dad-1* +; CF4, + *dad-4* +. In the above and other similar crosses described in the text, 96–128 transductants were scored.

induction at 40 °C will produce an enzyme which has been inactivated prior to harvesting the culture and will not be reactivated by subsequent incubation and assay at 28 °C. In the case of CF2ts4 the locus for the thermosensitive phenotype was cotransduced with *ara* and *leu* at about the same frequencies as the original *dad-2*. CF10ts3 was not mapped. The presence of mutations in *dad* which render the pre-formed D-alanine dehydrogenase activity thermosensitive indicate conclusively that *dad* is a structural gene for this enzyme.

The product of the *dad* gene was identified by polyacrylamide gel electrophoresis as a protein of molecular weight $55–60 \times 10^3$ d which was present in solubilized membrane preparations from alanine-induced AB259, but was absent from similar

Table 4. *Influence of temperature on induction and activity of* D-*alanine dehydrogenase in wild-type and temperature sensitive strains*

Strain and temperature of induction	Temperature of enzyme assay (°C)	Activity of enzyme
AB259 (30°)	28	84
	42	126
AB259 (42°)	28	68
	42	94
CF2ts4 (30°)	28	72
	42	34
CF2ts4 (42°)	28	55
	42	23
CF10ts3 (28°)	28	31
	40	9
CF10ts3 (40°)	28	4
	40	4

Cells were grown at 37 °C in succinate minimal medium, washed in buffer (37 °C), and induced by incubation with DL-alanine for 3 h at the temperatures indicated.

Table 5. *Specific activities of* D-*alanine dehydrogenase in Dad*$^+$ *revertants of GIA68*

	Specific enzyme activities from cells grown with carbon sources	
Strain	Glycerol	DL-alanine
KMBL146	3	80
GIA68	2	4
CF251	5	68
CF252	4	58
CF253	6	63
CF254	5	71
CF255	4	49

CF251-5 are Dad$^+$ revertants of GIA68 obtained as described in Materials and Methods.

preparations of the deletion mutant CF13. Proteins used as molecular weight standards were catalase (60×10^3 d), alcohol dehydrogenase (48×10^3 d) and trypsin ($23{\cdot}4 \times 10^3$ d).

(iii) *Revertants of GIA68* alnR

GIA68, the *alnR* mutant of Beelen *et al.* (1973) was phenotypically Dad$^-$ and the locus *alnR* was shown by these workers to have a regulatory function. The Dad$^-$ phenotype of GIA68 can be explained either in terms of a mutation which inactivates the co-inducer protein of a system with positive or positive–negative control, or in terms of a mutation which converts the repressor protein of a negative control system to a super-repressor. The question of whether the *alnR* product is a positive or negative controlling element is clarified by the remaining results of this paper.

5 independent Dad$^+$ revertants of GIA68 were isolated and tested for possession of, and inducibility of D-alanine dehydrogenase. The results in Table 5 show very clearly that in all 5 revertants D-alanine dehydrogenase activity has been restored. The basal enzyme levels are similar to that in wild-type and are inducible by alanine to 56–85 % of the fully induced wild-type level. This result is not consistent with the idea that the *alnR* phenotype in GIA68 results from the modification of a negative control element to form a super-repressor, because such mutations in the negatively controlled *lac* operon revert predominantly to constitutive phenotypes caused by relatively non-specific mutations which inactivate the regulatory protein (Willson *et al.* 1964).

(iv) *Isolation of constitutive mutations*

Several attempts were made to isolate constitutive D-alanine dehydrogenase mutants. The alternate subculture method with alanine/glucose, alanine/succinate and alanine/glycerol as pairs of alternate carbon sources (McFall, 1964) and the continuous culture method with alanine as growth-limiting nitrogen source (Bloom & McFall, 1975) were both used. No fully constitutive strains were isolated, however, a large number of low-level constitutive strains were obtained in which the constitutive level was 18–30 % of the fully induced level (the basal level in wild-type is 8–10 % of the fully induced level).

In the negative control *lac* system fully constitutive mutants arise frequently by inactivation of the regulator protein and are isolated rather easily (Jacob & Monod, 1961; Willson *et al.* 1964). Full constitutives arise less frequently but can be readily isolated from the strictly positive maltose and D-serine deaminase systems of *E. coli* (Hofnung & Schwartz, 1971; Bloom & McFall, 1975), whereas in the positive–negative control *ara* system low level constitutives are the rule (Englesberg *et al.* 1965). Therefore, our failure to obtain *dad* constitutive mutants argues against the operation of a negative control system, whilst the preponderance of low-level constitutives isolated favours the existence of a positive–negative system.

(v) *Complementation between* alnR *alleles*

For complementation studies it was initially intended to use the F factors F101 and F104. However, both strains obtained were phenotypically Dad$^-$, failing to utilize D or L-alanine as carbon source for growth. Further investigations (not reported here) indicated that these strains carry recessive *dad* mutations on both episome and chromosome.

As F101 and F104 were unsuitable for complementation studies with *alnR* we isolated, *de novo*, an F′ factor carrying the appropriate chromosomal region. This was achieved by mating AB259 (HfrH) with GIA68*recA* and selecting for rare Thr$^+$ Leu$^+$ Str^r clones. One of these clones, CF240, was shown to contain an F plasmid derivative by its sensitivity to F-specific phage MS2, the curability of the Thr$^+$ Leu$^+$ character, and its ability to transfer both these markers rapidly and at high frequency during conjugation with F$^-$ recipients. CF240 was able to grow on alanine, possessed normal levels of D-alanine dehydrogenase and was therefore phenotypically Dad$^+$. After treatment of CF240 with acridine orange, cured strains were selected by virtue of their Thr$^-$ Leu$^-$, MS2-sensitive phenotype. These strains (e.g. CF241) were phenotypically *Dad*$^-$. This result indicates conclusively that *alnR*$^+$ is fully *trans* dominant to *alnR*$^-$. As super-repressor mutations in the negative control *lac* system are dominant to the wild regulator allele (Jacob & Monod, 1961) whereas regulator–negative mutations in the positive–negative control *ara* system (Englesberg *et al.* 1965) or in the positive control D-serine deaminase system (Bloom *et al.* 1975) can be fully recessive to wild-type, our data indicate that *alnR* is a component of a positive–negative or positive control system.

4. DISCUSSION

Our results show that *alnA* and *dad* are alternative names for the same gene which codes for a structural component (molecular weight $55–60 \times 10^3$) of the enzyme D-alanine dehydrogenase. The position of *dad* on the *E. coli* linkage map was previously located between *ara* and *leu* at about minute 1·5 (Beelen *et al.* 1973; Franklin & Venables, 1976).

The results of other workers have implicated a different region of the chromosome in D-alanine dehydrogenase synthesis. Wild *et al.* (1973) described a D-amino acid dehydrogenase in *Salmonella typhimurium*, possession of which allowed histidine-requiring auxotrophs to utilize D-histidine as a source of this amino acid. Mutants defective in this enzyme were termed *dadA* and mapped near *hemA* on the *S. typhimurium* linkage map; study of a thermosensitive revertant identified *dadA* as a structural gene for the enzyme. The enzyme was later shown to be essential for the utilization of alanine isomers as sources of carbon and nitrogen for the growth of *S. typhimurium* (Wild *et al.* 1978) and appears therefore to be the analogous enzyme to the D-alanine dehydrogenase of *E. coli*. Mutations in *E. coli* which increased levels of a D-amino acid dehydrogenase and apparently released it from catabolite repression were mapped between *hemA* and *purB* at about 26

on the linkage map by Kuhn & Somerville (1971), while very recently, T. Klopotowski (personal communication) has isolated a number of D-amino acid dehydrogenase-less mutants of *E. coli* which fail to grow on alanine isomers and also map between *hemA* and *purB*. Similar mutants, but of a more complex nature have been isolated by Wijsman & Kruyt; their cotransduction with *pyrF* was of the order of 20% (strain GIA44, Wijsman & Kruyt, unpublished results).

It appears therefore that the structure of D-amino acid (alanine) dehydrogenase is controlled by two distinct, widely separated loci, *dad* (*alnA*) (1·5 min) and *dadA* (26 min). This conclusion is supported by the recent results of Olsiewski *et al.* (1980) which show that D-amino acid dehydrogenase of *E. coli* is composed of two subunits of molecular weights 45×10^3 and 55×10^3. The closeness of the latter figure to our molecular weight for the *dad* product suggests that the *dad* gene is the template for the larger subunit. It seems likely that the locus at 26 min will prove to be the template for the 45×10^3 subunit.

Our results show that *alnR*, the regulatory gene mapped by Beelen *et al.* (1973) at about minute 99 on the *E. coli* linkage map, regulates D-alanine dehydrogenase synthesis in a positive way. This is deduced from the *trans* dominance of $alnR^+$ to $alnR^-$ and the absence of constitutives among Dad^+ revertants of the $alnR^-$ mutation. The existence of a negative control system would require that $alnR^-$ produced a super-repressor and would therefore be *trans* dominant to $alnR^+$ and revert predominantly to constitutive Dad^+ by mutations which inactivate the *alnR* product completely (Jacob & Monod, 1961; Willson *et al.* 1964). Our failure to isolate fully constitutive mutants also supports the existence of a positive control system. Wild & Klopotowski (1975) drew the same conclusion from their failure to isolate D-amino acid dehydrogenase constitutives in *S. typhimurium*. Our results do not rule out the possibility that the *alnR* product has both positive and negative controlling functions as does the *araC* product (Englesberg *et al.* 1965).

The biochemical and genetic nomenclature of the D-alanine dehydrogenase system is currently complicated by the existence of alternative names and symbols bestowed by different authors. In the interest of simplicity and uniformity we make the following proposals: (i) that in view of its activity with amino acids other than D-alanine, the name D-amino acid dehydrogenase should be used for the enzyme and not D-alanine dehydrogenase; (ii) that the presumed structural gene near *hemA* be called *dadA* in conformity with its equivalent on the *S. typhimurium* chromosome; (iii) that the structural gene at minute 1·5 (currently *alnA* or *dad*) be called *dadB*; it then follows most logically that (iv) the known regulatory gene at about minute 99 (currently *alnR*) be called *dadQ* as the name *dadR* has already been used for the regulatory locus at about minute 26. The status of *dadR* is uncertain, but by analogy with its equivalent on the *S. typhimurium* chromosome it would seem likely to represent the promoter-operator sequence of *dadA*.

Note added in proof

J. Wild & T. Klopotowski (*Molecular and General Genetics* **181**, 373–378 (1981)) have very recently published the map position of a structural gene for D-amino

acid dehydrogenase of *E. coli*. This gene has been named *dadA* and maps between *hemA* and *purB*. These authors also state that (i) they have examined some of the *dad* mutants of Franklin & Venables (1976) and found they now fail to grow on pyruvate and have normal levels of D-amino acid dehydrogenase activity as measured by a different procedure from that used by Franklin & Venables (1976) and in the present paper; and (ii) that *E. coli* strains CSH73 and RFS889 both of which carry *leu-ara* deletions, retain the ability to utilise alanine isomers as carbon sources for growth. We wish to point out that Beelen *et al.* (1973) stated that their *alnA* mutant grew normally on pyruvate and that, although not published, this was also the observation of Franklin & Venables (1976) for their *dad* mutants. Additionally the *alnA* mutant was found to lack D-amino acid dehydrogenase activity when assayed by a procedure similar to that used by Wild & Klopotowski (1981). We have also found (unpublished results) that CSH73 grows on alanine but also that it can restore the ability of *dad* mutants to grow on alanine when used as a *P1* transduction donor. This suggests that it carries a transposition of the *dad* region.

REFERENCES

Beelen, R. H. J., Feldmann, A. M. & Wijsman, H. J. W. (1973). A regulatory gene and a structural gene for alaninase in *Escherichia coli*. *Molecular and General Genetics* **121**, 369–374.

Bloom, F. R. & McFall, E. (1975). Isolation and characterization of D-serine deaminase constitutive mutants by utilization of D-serine as sole carbon or nitrogen source. *Journal of Bacteriology* **121**, 1078–1084.

Bloom, F. R., McFall, E., Young, M. C. & Carothers, A. M. (1975). Positive control in the D-serine deaminase system of *Escherichia coli* K12. *Journal of Bacteriology* **121**, 1092–1101.

Churchwood, G. G. & Holland, I. B. (1976). Envelope synthesis during the cell cycle in *Escherichia coli* B/r. *Journal of Molecular Biology* **105**, 245–261.

Englesberg, E., Irr, J., Power, J. & Lee, N. (1965). Positive control of enzyme synthesis by gene C in the L-arabinose system. *Journal of Bacteriology* **90**, 946–947.

Franklin, F. C. H. & Venables, W. A. (1976). Biochemical, genetic and regulatory studies of alanine catabolism in *Escherichia coli* K12. *Molecular and General Genetics* **149**, 229–237.

Hofnung, M. & Schwartz, M. (1971). Mutations allowing growth on maltose of *Escherichia coli* K12 strains with a deleted *malT* gene. *Molecular and General Genetics* **112**, 117–132.

Jacob, F. & Monod, J. (1961). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology* **3**, 318–356.

Kuhn, J. & Somerville, R. L. (1971). Mutant strains of *Escherichia coli* K12 that utilize D-amino acids. *Proceedings of the National Academy of Science, U.S.A.* **68**, 2484–2487.

Lennox, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage *P1*. *Virology* **1**, 190–206.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin reagent. *Journal of Biological Chemistry* **193**, 265–275.

McFall, E. (1964). Pleiotropic mutations in the D-serine deaminase system of *Escherichia coli*. *Journal of Molecular Biology* **9**, 754–762.

Olsiewski, P. J., Kaczorowski, G. J. & Walsh, C. (1980). Purification and properties of D-amino acid dehydrogenase, an inducible membrane-bound iron–sulfur flavoenzyme from *Escherichia coli* B. *Journal of Biological Chemistry* **255**, 4487–4494.

Wild, J., Walczak, W., Grynkiewicz, K.-K. & Klopotowski, T. (1973). D-amino acid dehydrogenase: the enzyme of the first step of D-histidine and D-methionine racemization in *Salmonella typhimurium*. *Molecular and General Genetics* **128**, 131–146.

Wild, J. & Klopotowski, T. (1975). Insensitivity of D-amino acid dehydrogenase synthesis to

catabolic repression in *dadR* mutants of *Salmonella typhimurium*. *Molecular and General Gentics* **136**, 63–73.

Wild, J., Filutowicz, M. & Klopotowski, T. (1978). Utilization of D-amino acids by *dadR* mutants of *Salmonella typhimurium*. *Archives of Microbiology* **118**, 71–77.

Willson, C., Perrin, D., Cohn, M., Jacob, F. & Monod, J. (1964). Non-inducible mutants of the regulator gene in the 'lactose' system of *Escherichia coli*. *Journal of Molecular Biology* **8**, 582–592.