MAPPING OF THE D-SERINE DEAMINASE REGION IN *ESCHERICHIA COLI* K-12

ELIZABETH McFALL[1]

Department of Microbiology, New York University School of Medicine, New York, New York 10016

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D-serine deaminase is an inducible enzyme in most strains of *E. coli*, and is also subject to catabolite repression (PARDEE and PRESTIDGE 1955). D-serine is bacteriostatic to *E. coli;* strains that cannot form the enzyme (*dsdA* mutants) do not multiply in minimal media containing D-serine (McFALL 1964a). It is therefore quite easy to score for the marker *dsdA*$^+$ among recombinants of genetic crosses. In previous experiments, *dsdA* was mapped near *serA* on the K-12 linkage map. Presumably this marker specifies the structure of the enzyme, as it is the only locus in which mutations resulting in a D-serine sensitive phenotype have arisen.

Several mutations that affect the regulation of D-serine deaminase synthesis have been isolated, and all were found to be closely linked to the *dsdA* locus. In order to study these mutations more closely at the genetic level, it was desirable to determine their exact location, relative to known markers. In the work presented below, the *dsdA* marker and the regulatory mutations affecting its expression are shown to be cotransducible with several known markers between *serA* and *his*.

MATERIALS AND METHODS

Nomenclature: Certain marker designations have been altered to accord with the rules suggested by DEMEREC, ADELBERG, CLARK and HARTMAN (1966). *dsdA*$^+$ and *dsdA:* genetic markers for ability or inability to form D-serine deaminase. Previous designation: *Dsd*$^+$ and *Dsd*$^-$. *dsdC*$^+$ and *dsdC:* genetic markers for inducible or constitutive formation of D-serine deaminase. Previous designation: *i* and *C*. (McFALL 1964a).

Bacterial strains and transducing phage: The bacterial strains utilized in this study are described in Tables 1 and 2. All are K-12 derivatives. Derivatives of strains W 3828 and AB 444 that harbor mutations affecting D-serine deaminase synthesis are described in Table 2. Strain AT1378 originally grew poorly, but a *try*$^+$ recombinant from a mating of AB311 (Hfr) $\times$ AT1378 was found to grow well. Therefore, only the recombinant, EM3000, was used in this work. A streptomycin resistant derivative of AT2022, EM3001, isolated by MR. K. BROWN, was used in the mapping of *pheB*. Phage P1kc (FRANKLIN and LURIA 1961), provided by DR. N. FRANKLIN, was used for transductions.

Media: Minimal medium contains per liter H_2O: 13.6 g KH_2PO_4, 0.5 g $(NH_4)_2\ SO_4$, 0.5 g NH_4Cl, 10 g glycerol, 40 mg $MgSO_4 \cdot 7H_2O$ (M/6000), 0.5 mg $FeCl_3$, and is adjusted to pH 7.0 with NaOH. Amino acids or purines are added as required to a final concentration of 50 mg/l, thiamine to a final concentration of 5 mg/l. Where lactose replaced glycerol as carbon source, it was used at final contentration 1%. D-serine (DS) was used at final concentration 500 mg/l, streptomycin at final concentration 200 mg/l. Minimal agar contains 15 g/l Bacto agar. LB

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TABLE 1

Primary list of bacterial strains

Strain No.	Sex	*str*	*cysC*	*pheB*	*dsd*	*aroB*	*aroC*	*purC*	*his*	*pro*	*lac*	*thi*	*arg*	Other
KL16	Hfr	S	+	+	+	+	+	+	+	+	?	+	+	.....
AB311	Hfr	R	+	+	+	+	+	+	+	+	—	—	+	*thr, leu*
AB444	F⁻	R	+	+	+	+	—	—	+	+	—	—	—	.....
AB1320	F⁻	S	+	+	+	—	+	+	—	—	—	—	+	.....
AT2022	F⁻	S	+	—	+	+	+	+	—	—	?	—	+	.....
AT2465	F⁻	S	+	+	+	+	+	+	+	+	?	..	+	*guaA*
AT1378	F⁻	?	—	+	+	+	+	—	+	+	—	—	—	*try*
2320-16	F⁻	?	+	+	+	+	+	+	+	+	+	+	+	.....
W3828	F⁻	R	+	+	+	+	+	+	+	+	—	+	+	.....

Symbols designating growth factor requirements, sugar utilization, and response to D-serine or streptomycin (+ indicates prototrophy, ability to utilize sugar, or ability to form D-serine deaminase, — indicates lack of same) are as follows: *cys*–cysteine, *phe*–phenylalanine, *dsd*–D-serine deaminase formation, *aro*–phenylalanine and tyrosine, *pur*–purines, *his*–histidine, *pro*–proline, *lac*–lactose utilization, *thi*–thiamine, *arg*–arginine, *thr*–threonine, *leu*–leucine, *gua*–guanine, *try*–tryptophan. strS and strR refer to sensitivity or resistance to 200 ug/ml streptomycin. The "genotypes" are given as + or —, R or S, because in most cases the mutation site is not known to the author. I wish to thank the donors of these strains, who were as follows: KL16, DR. B. LOW; AB311, DR. G. S. STENT; AB444, AB1320, AT2022, AT2465, DR. A. L. TAYLOR; AT1378, DR. A. J. CLARK, 2320-16, DR. J. BECKWITH; W3828, DR. P.H.A. SNEATH.

broth was formerly referred to as L-broth (LURIA, ADAMS and TING 1960), LB agar is LB broth supplemented with 1.1% agar.

Bacterial matings and transductions: Hfr and F⁻ strains were grown and mated in LB broth. The initial concentrations of donor and recipient in mating experiments were respectively about 5×10^7 and 5×10^8 bacteria/ml. Matings were interrupted at specified intervals after appropriate dilution by the method of Low and WOOD (1965), and plated on selective media for scoring of recombinants. Preparation of transducing phage and transductions were described previously (MCFALL 1964a). Preparations of transducing phage were always harvested at the time of mass lysis of the infected culture, 4 to 5 hours after infection.

D-serine deaminase assay: The D-serine deaminase assay was performed as described previously (MCFALL 1964a), except that only 0.3 ml of cell suspension was used for the assay, 0.05 ml of toluene was added to it, and this mixture was incubated for 15 min at 37° before the addition of D-serine. These modifications were found to result in greater reproducibility of results.

TABLE 2

Mutants with altered dsd *phenotype*

Stock number	*dsd* genotype	Previous designation	Mutagen	Phenotype
EM3003	*dsdA7*	AB444 *Dsd*α^-	UV	negative
EM1101	*dsdA1dsdC1*	W3828 *Dsd*$^-$*C1*	UV	negative
EM1100	*dsdC1*	W3828 *Dsd*$^+$*C1*	spontaneous	low constitutive
EM1200	*dsdC2*	W3828 *Dsd*$^+$*C2*	EMS	full constitutive
EM1300	*dsdC3*	W3828 *Dsd*$^+$*C3*	EMS	low constitutive, catabolite sensitive
EM1400	*dsdC4*	W3828 *Dsd*$^+$*C4*	EMS	low constitutive, catabolite sensitive
EM1500	*dsdC5*	W3828 *Dsd*$^+$*C5*	EMS	full constitutive
EM1600	*dsdC6*	W3828 *Dsd*$^+$*C6*	EMS	full constitutive

UV: ultraviolet light; EMS: ethyl-methanesulfonate.

RESULTS AND DISCUSSION

Gene order in the dsd *region:* The *dsd* marker was previously located between *serA* and *his* on the K-12 linkage map (McFALL 1964a). In order to determine its exact location, attempts were made to demonstrate cotransduction of it with known markers. Stocks of the transducing phage P1kc were prepared on substrains of W3828 which either carry *dsdA1* or *dsdC1*, and on strain AT2465, which carries *guaA*. These phage stocks were used to transduce strains carrying mutations in *aroC*, *purC*, *aroB*, *cysC*, *guaA* and *pheB* to prototrophy for these markers, and the transductants were then scored for inheritance of *dsd* or *gua* alleles. The results are presented in Table 3. It may be seen that *dsdA*, and its constitutivity determinant *dsdC1*, are closely linked to *aroB*, *aroC*, and *purC*, and that *dsdC1* is apparently closer to *purC* than is *dsdA*. Phage P1 grown on strain AB1320 (*aroB*) could transduce strain AB444 carrying (*aroC purC*) to Pur$^+$ with normal frequency, but failed to transduce the same strain to Aro$^+$. It seemed indicated, therefore, that *aroC* and *aroB* are quite close. PITTARD and WALLACE (1966) have shown that in fact the two mutations affect the same gene.

The order among the markers *dsdA*, *aroC*, and *purC* can be established by determining the proportions of the various types of recombinants that emerge from a transduction in which the donor strain carries *dsd*$^+$ *aro*$^+$ *pur*$^+$ and the recipient, *dsdA aroC purC* mutations. Such a transduction was performed, with strain EM1100 as donor, and strain EM3003 as recipient. *dsd*$^+$, *aro*$^+$, and *pur*$^+$ were selected in individual transductions, and the other two markers were then scored. The results are presented in Table 4. It may be seen that the order is unambiguously established as *dsdA-arcC-purC*, with *dsdA* and *purC* each showing about 50% cotransduction with *aroC*, and showing 25% joint cotransduction with *aroC*. There are some discrepancies in cotransduction frequencies, depending on which marker is selected; these will be discussed below.

No cotransduction was found among the markers *guaA*, *cysC*, *pheB*, and *dsdA1*. This last finding was disappointing, since mating experiments had indicated that

TABLE 3

Cotransduction among markers in the dsd *region*

Donor strain	Recipient strain	Marker selected	No. of transductants	Unselected marker scored	No. of cotransductants	Percent cotransdutcion
EM1100	AB444	*aro*+	274	*dsdC1*	154	56
	AB444	*pur*+	120	*dsdC1*	41	34
	AB1320	*aro*+	64	*dsdC1*	30	49
EM1101	AB444	*pur*+	130	*dsdA1*	28	22
	EM3000	*cys*+	132	*dsdA1*	0	0
	AT2022	*phe*+	128	*dsdA1*	0	0
AT2465	EM1101	*dsd*+	320	*guaA*	0	0
	EM3000	*cys*+	360	*guaA*	0	0
	AT2022	*phe*+	200	*guaA*	0	0

P1 phage grown on the strains indicated was used to transduce recipient strains as indicated. Transductants were patched to master plates, and cotransduction of unselected markers was then scored by replica plating from the master plates.

TABLE 4

Cotransduction of dsdA, aroC, *and* purC

Marker selected	No. of transductants	Unselected marker scored	No. of cotransductants	Percent cotransduction
dsd+	89	*aro*+	22	25
		pur+	11	12
		aro+*pur*+	7	8
aro+	100	*dsd*+	49	49
		pur+	56	56
		dsd+*pur*+	25	25
pur+	63	*aro*+	53	84
		dsd+	13	21
		aro+*dsd*+	6	10

P1 phage grown on strain EM1100 was used to transduce strain EM3003 to *dsd*+, *aro*+, or *pur*+. Transductants were patched to master plates, and cotransduction of unselected markers was then scored by replica plating from the master plates.

these markers are fairly closely linked (TAYLOR and THOMAN 1964). However, it must be noted that the mapping was by time of entry from strain AB313, whose origin of transfer is near *met*, a considerable distance from the *dsd* region, and the actual positions of the genes may not be exactly as the timing suggested.

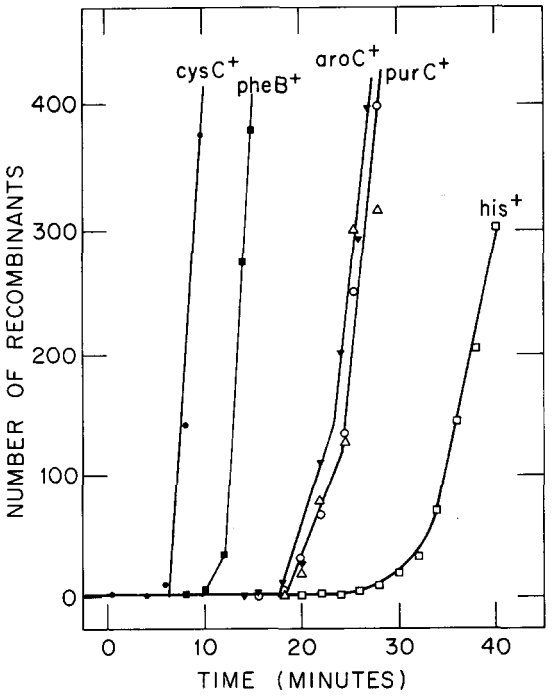

FIGURE 1.—Kinetics of transfer of various markers in the *dsd* region by the Hfr strain KL16. The ordinate scale is the interval between the time at which the parental cultures were mixed (time zero) and the time at which the sample was blended to interrupt mating.

Moreover, through a misunderstanding, *dsd* was placed to the right of *aroC* and *purC* on the published map; it actually lies to the left of *aroC*.

Low (1965) has recently isolated an Hfr with origin of transfer at *lys*. This Hfr, KL16, was used to map *cysC*, *aroC*, *purC*, and *pheB* independently in the F^- strains AB444, EM3000, and EM3001. AB444 contains both *aroC* and *purC*, EM3000 both *cysC* and *purC*, EM3001 both *pheB* and *his4*. The results are presented in Figure 1. If a uniform subtraction of 4 min (the approximate time required to transfer Origin [Low 1965]) is applied to each time of entry, it may be seen that *cysC* is transferred at 2 minutes, which places it near *galR*, a little to the left of its previously assigned position, and fairly close to its counterpart on the Salmonella linkage map (SANDERSON and DEMEREC 1965). *aroC* enters at 13.5 min, just before *purC*, which places it a little to the right of its previous position and very close to *aroB*, in agreement with the transduction studies described above. *purC* is transferred to both strains at 14.0 min, which places it also to the right of its former position. *pheB* enters at 6 min, about at *tyr*, in agreement with the findings of PITTARD and WALLACE (1966). These corrections are relatively small, but they explain the lack of cotransduction between markers that had appeared to be closely linked. It may be noted that his^+ is not transferred to strain EM3001 until 26 min, somewhat later than the map predicts. TAYLOR and THOMAN (1964) also observed late entry of his^+ with the streptomycin sensitive parent of this strain, AT2022; possibly genome transfer into this strain is slower than into others.

Several genes have been mapped in the *dsd* region since publication of the TAYLOR and THOMAN map, and one of them *supN23* EGGERTSSON and ADELBERG (1965), has been shown by G. EGGERTSSON (personal communication) to lie slightly to the left of *dsdA*. EGGERTSSON has found the two genes to be cotransduced with a frequency of 23%. A map of the *lys-his* region, showing the corrections and the addition of *supN23*, is presented in Figure 2.

Mapping of mutations affecting regulation of D-*serine deaminase synthesis:* Six mutants that form D-serine deaminase constitutively (*dsdC*) have been isolated from strain W3828. Two of the mutations also affect catabolite repressibility of enzyme synthesis; these mutations lie closer to the *dsdA* gene than do the other four (MCFALL 1964b). These *dsdC* mutations can be tentatively ordered relative

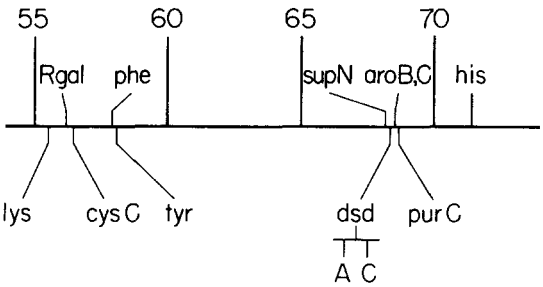

FIGURE 2.—Revised map for some genes in the *dsd* region. Numbers above the interval markings refer to time of entry positions on the map of TAYLOR and THOMAN (1964).

to *aroC* and *dsdA* by two-point crosses. As has been shown above, *dsdA7* is 49% linked to *aroC*, and 21% linked to *purC*. The *dsdC* markers have been mapped relative to *aroC* by transducing strain 444 to *aro*$^+$, sometimes also to *pur*$^+$, with stocks of phage P1 grown on the various *dsdC* mutants (Table 2). The proportion of constitutive cotransductants is then a measure of the order between *aroC* and the *dsdC* marker carried by the donor strain. The results, which indicate the order to be *dsdA7-dsd [C3-C4 (C2, C5, C6) — C1] — — — aroC*, are presented in Table 5. This order is uncertain to the extent defined by the sampling error (95% limit), and it is also conceivable that the various *dsd* mutations themselves influence the probabilities of their cotransduction with *aroC*. The limits set by the sampling error do show that unless disparate influences exist, *dsdC3* and *dsdC1* belong to different classes, such that *dsdC1* is closer to *aroC* than is *dsdC3*. *dsdA7* could belong to either class, or to neither. Moreover, previous observations on cotransduction of the various *dsdC* markers with *dsdA*, and frequencies of inducible recombinants in reciprocal transductions involving these markers, had indicated the same order of *dsdC* markers relative to *dsdA*, (McFALL 1964a), and strengthen confidence in the validity of these data. It may be noted that *dsdC3* appears to be to the left of *dsdA7* since its cotransduction frequency with *aro*$^+$ is lower than that of *dsdA7*. The amount of possible error due to sampling, however, is large enough that this result may have occurred by chance and may not indicate the true map positions. In previous experiments no inducible recombinants were found in transductions between *dsdC3* and *dsdC4*, and sufficiently few between these and the other *dsdC* mutations to indicate that all the *dsdC* markers are on the same side of the *dsdA* gene. It is, of course, not excluded that *dsdC3* is a mutation in the *dsdA* gene itself, or that some of the *dsdA* mutations are regulatory mutations that result in loss of enzyme forming capacity.

Complicating factors in transduction studies: Some points came up in the course of this work which, while they do not relate directly to the D-serine deaminase system, may still be of interest in regard to the general use of transduction studies.

TABLE 5

Cotransduction of dsdA7 *and various* dsdC *markers with* aroC

Donor strain	Recipient strain	Marker selected	No. of transductants	Unselected marker scored	No. of cotransductants	Percent of cotransduction		Sampling error
EM1100	AB444	*aro*+	274	*dsdC1*	154	56.5	±	5.9
EM1200	AB444	*aro*+	157	*dsdC2*	84	53.5	±	7.8
EM1300	AB444	*aro*+	189	*dsdC3*	73	38.6	±	6.7
EM1400	AB444	*aro*+	76	*dsdC4*	35	46.1	±	11.4
EM1500	AB444	*aro*+	133	*dsdC5*	66	49.6	±	8.6
EM1600	AB444	*aro*+	155	*dsdC6*	75	48.3	±	7.6
W3828	EM3003	*aro*+	465	*dsdA7*+	225	48.4	±	4.5

P1 phage grown on the strains indicated was used to transduce recipient strains as indicated. To score cotransduction of *C* markers, transductant colonies were picked into minimal medium supplemented with arginine, adenine, and thiamine, and cultivated overnight in test tubes at 35° with shaking. The cells were then harvested and assayed for enzyme content. To score cotransduction of *dsd*+ with *aro*+, *aro*+ transductants were patched to master plates, these were then replicated o plates containing D-serine. The sampling error is two standard deviations, $1.96\ (xy/N)^{1/2}$ where x is the fraction of cotransductants, $y=1-x$, and N is the total number of transductants.

(1) *Heterogeneity in transducing phage:* One may ask whether the class of P1 transducing particles that carries a given marker is homogenous or heterogeneous with regard to other markers. Stocks of the Salmonella transducing phage, P22, appear to be heterogeneous (PEARCE and STOCKER 1965; ROTH and HARTMAN 1965). This problem can be examined using strain EM1600 as donor and strain AB444 as recipient, and comparing the frequency of cotransduction of *dsdC6* when both *pur*$^+$ and *aro*$^+$ are selected to the frequency of cotransduction of *dsdC6* with either *aro*$^+$ or *pur*$^+$ alone. (As will be shown below, nearly all P1 particles which can effect transduction of *pur*$^+$ must also carry *aro*$^+$, hence error introduced into such a comparison by the exclusion of the class of P1 particles that carries *pur*$^+$, but not *aro*$^+$, would be negligible). When *aro*$^+$ alone is selected, the mean frequency of cotransduction of the *dsdC* marker is 51% (264 *dsdC6* colonies of 515 total *aro*$^+$ transductants). If *pur*$^+$ alone is selected, the mean frequency of cotransduction of *dsdC* is much lower, 36.8% (88 *dsdC6* colonies of 239 *pur*$^+$ transductants). This gradient could presumably be attributable either to heterogeneity in the contents of the transducing particles, or to decreasing probability of integration of the unselected marker as the distance between it and the selected marker increases, or both. If it were due to the second cause alone, the selection of *aro*$^+$ together with *pur*$^+$ should yield a higher proportion of transductants carrying *dsdC* than the selection of *pur*$^+$ alone. If it were due primarily to heterogeneity in the phage, the added selection of *aro*$^+$ should have little effect, the frequency of cotransductants carrying *dsdC6* would be fixed by the selection of the more distal marker, *pur*$^+$. In fact, when *aro*$^+$ and *pur*$^+$ both were selected, the frequency of cotransduction of *dsdC6* was 35.3% (118 *dsdC6* colonies of a total of 334 *aro*$^+$ *pur*$^+$ transductants), the same as its cotransduction frequency with *purC* alone. Thus it seems indicated that those P1 transducing phage which carry markers in the *dsd* region are heterogeneous, since not all of those which carry *aro*$^+$ carry *pur*$^+$ as well.

(2) *Lack of reciprocity in cotransductions:* During the course of this work it was observed that the values obtained for frequency of cotransduction of two linked markers in the *dsd* region depends on which of the two is selected. Other workers, including G. EGGERTSSON (personal communication) have made similar observations. Thus, when *aro*$^+$ is the selected marker with W3828 as donor and EM3003 as recipient, the frequency of cotransduction of the unselected marker *pur*$^+$ is 50% (Table 6). If *pur*$^+$ is the selected marker and *aro*$^+$ the unselected

TABLE 6

Reciprocal cotransduction frequencies for linked markers dsdA, aroC, *and* purC

Marker selected	No. of transductants	Marker scored	No. of cotransductants	Percent cotransduction		Sampling error
dsd+	513	*aro*+	168	32.8	±	3.9
aro+	465	*dsd*+	225	48.4	±	4.2
aro+	1077	*pur*+	541	50.2	±	3.0
pur+	287	*aro*+	220	79.5	±	4.4

marker, however, the frequency of cotransduction is 80%. There is also lack of reciprocity, though it is less striking, between *dsdA7* and *aroC* in this experiment. It may be recalled (Table 4) that in the recipient strain EM3003 the frequency of cotransduction of *dsd*$^+$ when *pur*$^+$ is selected is about double the frequency of cotransduction of *pur*$^+$ when *dsd*$^+$ is selected.

Finally, it has been observed by many workers, and also in this laboratory, that all markers are not transduced with equal frequency. For markers studied during the course of these experiments, the frequencies of transduction arrange themselves in the ratios *cys:lac:arg:dsd:aro:ade* of 7:4:4:2:2:1 (Table 7). Although the *lac* phenotype of strain EM1011 is due to a considerable deletion in the *z* gene (*lac11D3*, COOK and LEDERBERG 1962), and *dsdA1* is a point mutation, *lac*$^+$ transductants are twice as frequent as *dsd*$^+$ transductants. *purC* may be a deletion, as it never reverts. *dsdA7*, *dsdA1*, and *aroC* all revert, though at frequency less than 10^{-8}.

The data of Table 6 would suggest that there is a polarity of integration from *purC* to *dsdA*. However, much of this apparent polarity can be explained by the relatively low frequency of *pur*$^+$ transductants. These are only half as common as *aro*$^+$ or *dsd*$^+$ transductants. Thus, it is more likely that a transducing phage capable of giving rise to *pur*$^+$ carries *aro*$^+$ than that an *aro*$^+$ phage carries *pur*$^+$.

The cause of the variation in frequency of transduction of markers is unknown. It may be that the phage is more prone to incorporate certain areas of the bacterial genome than others. This would not be surprising. However, it is also conceivable that the molecular nature of a mutation itself, or of a neighboring mutation, affects the probability of complete transduction of the wild-type allele. Thus, if *purC* is a deletion, perhaps the *pur*$^+$ transducing piece must carry *aro*$^+$ in order for pairing and integration of *pur*$^+$ to occur. Such effects at the molecular level might also explain the inequality in reciprocal transduction frequency between *dsdA7* and *aroC*. In this connection, detailed studies of RAVIN and IYER (1962) on transformation of erythromycin resistance in Pneumococcus indicate that mutant configurations may strongly influence recombination frequency.

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TABLE 7

Frequencies of appearance of various markers

Donor	Recipient	*ade*$^+$	*aro*$^+$	*arg*$^+$	*dsd*$^+$	*aro*$^+$ *ade*$^+$	*lac*$^+$	*cys*$^+$
W3828	AB444	118	289	...	...	73	...	...
W3828	EM3003	684	...	...	1008	...	...	...
W3828	EM3003	917	...	4083	1930	...	...	...
W3828	EM3003	85	127	...	177	...	...	...
W3828	EM3000	126	...	...	...	...	...	800
2320–16	EM1101	...	...	...	1826	...	3169	...
2320–16	EM3033	417	...	1579	617	...	...	...

SUMMARY

dsdA, a gene specifying synthesis of D-serine deaminase in *E. coli* K-12, *dsdC*, a region concerned with its metabolic control, and several other genes in the *dsd* region have been mapped relative to *lys* and *his* by interrupted mating and transduction. Certain anomalies encountered in transduction studies have been noted and discussed.

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