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Transfer of a Gene for Sucrose Utilization into *Escherichia coli* K12, and Consequent Failure of Expression of Genes for D-Serine Utilization

By N. GÜRDAL ALAEDDINOGLU* AND H. P. CHARLES

Department of Microbiology, The University, Reading RG1 5AQ

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As the first stage in investigating the genetic basis of natural variation in *Escherichia coli*, the gene(s) conferring the ability to use sucrose as a carbon and energy source (given the symbol *sac*$^+$) was transferred from a wild strain to K12, which does not use sucrose. The *sac*$^+$ region was transferred by two different methods. On both occasions it took a chromosomal location at minute 50·5 on the linkage map, between *aroC* and *supN*, in the region of the *dsd* genes, which confer the ability to use D-serine as a carbon and energy source. When the *sac*$^+$ region was present in the K12 chromosome the bacteria were unable to use D-serine as a carbon and energy source. In F'*sac*$^+$/*dsd*$^+$ diploids, the *dsd*$^+$ genes were similarly not expressed. Strain K12(*sac*$^+$) bacteria were sensitive to inhibition by D-serine; they mutated to D-serine resistance with much greater frequency than did a *dsd* mutant of K12. Such bacteria also mutated frequently to use raffinose. Strain K12(*sac*$^+$) bacteria did not utilize sucrose when they carried a mutation affecting the phosphotransferase system.

INTRODUCTION

This report describes the first stage of an investigation into the genetic basis of the variation shown by wild strains of *Escherichia coli*. The character chosen for analysis was the ability to use sucrose as a carbon and energy source. The method envisaged was to introduce the chosen character from a wild strain into the laboratory strain K12 and to examine whether the introduced genes became integrated into the chromosome. For ease of manipulation it was necessary that the chosen character should be easily selectable, and that K12 should be naturally negative for the character. It was also necessary that the character should be specified by one gene or cluster: a more complex character might depend upon genes dispersed about the chromosome, and transfer and analysis of the character would then be difficult.

A consideration in the choice of the character was that about 50% of isolates of *E. coli* are sucrose-positive (Edwards & Ewing, 1972); Edwards & Ewing's data relate to the proportion of isolates which ferment sucrose, but the ability to ferment a sugar is commonly correlated with the ability to use it as a carbon and energy source. Many characters of *E. coli* show a skewed distribution, in that the majority of isolates are positive or negative. When a small proportion of isolates are positive for a character there is probably a fair chance that the character may be conferred by a plasmid. For example, it is known that H_2S production by some *E. coli* strains (Ørskov & Ørskov, 1973) and lactose fermentation by some *Salmonella typhi* strains (Baron *et al.*, 1959) are due to plasmids. It seems less likely that a character for which 50% of strains are positive may be due to plasmids, and it is improbable that all variation in *E. coli* is caused by plasmids. In this investigation special importance was

* Present address: Middle East Technical University, Ankara, Turkey.

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attached to finding and studying a character showing natural variation in chromosomal genes. The supposition was that variation due to differences in chromosomal genes might reflect less ephemeral selective effects in nature than variation due to the presence and absence of plasmids. The present paper shows that ability to use sucrose is a chromosomal character. In a subsequent report it will be shown that linked genes are necessary for *E. coli* to use sucrose, but in the present report the genes involved will be referred to as the sucrose 'gene' and will be represented by the symbol *sac*.

METHODS

Media. Glucose minimal medium was medium E of Vogel & Bonner (1956) supplemented with thiamin (5 μg ml^{-1}) and glucose (5 g l^{-1}). When necessary, glucose was replaced by other carbon and energy sources at the same concentration unless stated otherwise. D-Serine was from Sigma. Minimal medium was supplemented with growth factors (40 μg ml^{-1}) and streptomycin (200 μg ml^{-1}) as required. Complete medium contained (g l^{-1}): Tryptone (Oxoid), 10; yeast extract (Difco), 5; K_2HPO_4, 3; KH_2PO_4, 1; glucose, 5. T_2 nutrient broth contained (g l^{-1}): nutrient broth base (Difco), 8; NaCl, 4. Dehydrocholic acid/neutral red agar (Morse & Alire, 1958) contained (g l^{-1}): Tris (pH 7·9), 1·3; dehydrocholic acid, 1·5; yeast extract, 1; Proteose Peptone (Difco), 10; neutral red, 0·075. When required, media were solidified with Difco Bacto-agar (10 g l^{-1}), autoclaved separately (5 g in 100 ml water). Soft T_2 nutrient agar for overlays contained 6 g agar l^{-1}, autoclaved in the medium.

Transduction. Phage P1kc was used. To prepare lysates, 0·03 ml 0·1 M-$Ca(NO_3)_2$ and 10^8 phage (0·1 ml) were added to 2 ml overnight broth culture and 15 min were allowed for adsorption; then 4 ml molten soft agar was added and the mixture was poured over 75 ml T_2 nutrient agar in a Petri dish (10 × 10 cm). Lysates were assayed on strain AB1621. For transduction, 10^9 phage (0·1 ml) and 0·03 ml 0·1 M-$Ca(NO_3)_2$ were added to 10^9 bacteria in 2 ml overnight broth culture and 12 min were allowed for adsorption; the suspension was then washed and resuspended in 1 ml minimal medium and 0·1 ml volumes were plated on selective media. Transductants were purified by restreaking before testing for the presence of unselected donor markers.

Conjugation. Bacteria were grown in complete medium to a density of 5×10^8 ml^{-1}; 0·5 ml of donor culture was then mixed with 9·5 ml of recipient culture, after plating control samples. In time-of-transfer experiments, the conjugating bacteria were separated by agitating for 30 or 60 s by the method of Low & Wood (1965).

Gene symbols and map distances. These follow Bachmann *et al.* (1976). The strains used are shown in Table 1. Except in Table 1, genes are shown in their order on the linkage map, starting at min 0. By agreement with Dr Bachmann the gene for sucrose utilization has been given the symbol *sac*$^+$, and is shown in parentheses when reference is to K12 strains, to indicate that the gene was introduced.

Induction of mutations. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (30 mg l^{-1}) was used by the method of Adelberg *et al.* (1965). For ultraviolet irradiation, a Camag Universal lamp, type T6-900, was used: 10^9 bacteria were resuspended in 10 ml minimal medium in a glass Petri dish, irradiated for 60 s to kill about 95 % of the bacteria and then incubated overnight in complete medium to allow expression of mutations. When appropriate, mutants were selected by the penicillin–cycloserine method of Ornston *et al.* (1969).

Curing bacteria of plasmids. The method of Salisbury *et al.* (1972) was used. To 100 ml nutrient broth, 1 g sodium dodecyl sulphate (SDS) was added; the solution was autoclaved, the pH value was adjusted to 7·6, and the solution was steamed for 1 h. Control solutions, without SDS, were prepared in the same manner. Overnight stationary-phase cultures in broth were diluted 100-fold and 0·5 ml volumes were added to 100 ml volumes of the SDS solution and control solution. The suspensions were incubated at 37 °C with shaking. When SDS was to be added to a growing culture, similarly prepared SDS solution (10 %, w/v) was added to bring the final concentration to 1 % (w/v).

RESULTS

Transfer of the ability to use sucrose to strain K12

Strain K12 did not use sucrose as a carbon and energy source but many isolates from nature did. Of the strains used in the present experiments, only K12 was sensitive to phage P1kc. At the time when the experiments were begun, the method of Goldberg *et al.* (1974) for selecting P1-sensitive variants of wild strains had not been published. The method used

Table 1. *Escherichia coli strains*

Strain	Genotype or derivation	Reference or source
AB265	F⁻ *thiA his leu thr argG lacY rpsL mtl xyl malA ara gal* λ^r λ^-	See Bachmann (1972)
AB1515	F⁻ *thiA mtl xyl mal trp purF lacY proC leu rpsL*	E. A. Adelberg
AB1621	F⁻ *lacY thiA mtl xyl galK ara rpsL tsx* λ^+	Adelberg *et al.* (1965)
EM1600	F⁻ *thiA dsdO lacZ supE rpsL*	McFall (1967*a*)
EM3000	F⁻ *thiA cysC purF argH xyl malA ara? gal tonA tsx rpsL supE?* λ^r, λ^-	McFall (1967*a*)
EM3003	F⁻ *thiA argH aroC purF dsdA7 tsx*	McFall (1967*a*)
JC5466	F⁻ *his trp rpsE recA56*	R. A. Dixon
FF8040	F⁻ *proC ptsI rpsL*	Epstein *et al.* (1970)
AB312	Hfr *thiA thr leu rpsL*	Taylor & Adelberg (1960)
AB347	Hfr *thiA thr leu aroC lacZ ara rpsL* λ^-	Huang & Pittard (1967)
AT997	Hfr *thiA rel dapC* λ^-	Bukhari & Taylor (1971)
AB2547	Hfr *ilvD argH purF xyl supN supE44*	Eggertsson & Adelberg (1965)
AB2575	Hfr *ilv thiA tsx*	E. A. Adelberg
GA121	Nalidixic acid-resistant mutant of AB1621	
GA122	*sac*⁺ transductant* of GA121	
GA301	*cys*⁺ *purF aroC* transconjugant from conjugation between AB347 and EM3000	
GA515	*sac*⁺ transductant* of AB1515	
GA501–509	Sucrose-utilizing hybrids between GAE10 and GA121; see text	
GA601–610	Strains of JC5466 carrying F′*sac*⁺ plasmids derived from a *sac*⁺ transductant* of AB312	
GAE10	Sucrose-utilizing isolate from the River Kennet, obtained by A. T. Regnier; carries transmissible antibiotic resistance	

* *sac*⁺ transductants were derived from the indicated parent using an R⁻ derivative of GA501 as donor.

for transferring genes in the present experiments was to irradiate sucrose-positive strains which contained R factors and select for transfer of the sucrose character to K12. The method gave transfer of the character to K12, but only in one of several experiments. The events leading to transfer of the genes for sucrose utilization are not understood, but the final result proved to be the same when a different method was used, as described below. The same results were also obtained when the method of Goldberg *et al.* (1974) was used (unpublished results).

The wild strains chosen as donors carried transmissible R factors but were sensitive to inhibition by nalidixic acid and streptomycin. The recipient strain, GA121, was resistant to nalidixic acid and streptomycin. The successful procedure was as follows. Late exponential-phase bacteria of GAE10 and other wild strains were resuspended (5×10^8 bacteria ml^{-1}) in minimal medium without glucose, exposed to ultraviolet radiation to kill 90%, held in the dark for 20 min, and then diluted 10-fold in complete medium (37 °C) and incubated. When the numbers of bacteria had reached about 5×10^8 ml^{-1}, the cultures were mixed with equal volumes of culture of GA121 (5×10^8 bacteria ml^{-1}) and incubated for 18 h. The mixtures of bacteria, and unmixed controls, were resuspended in minimal medium, plated on to minimal agar medium supplemented with sucrose (1%), nalidixic acid (25 mg l^{-1}) and streptomycin (500 mg l^{-1}) and incubated for 2 d. Ten experiments of this kind were made, using the same parental strains but different doses of radiation and different concentrations of antibiotics. Only one experiment, using strain GAE10, was successful, and gave nine transconjugants which were numbered GA501 to 509.

The characters of the transconjugants showed that they were GA121 bacteria which had acquired the ability to utilize sucrose and the ability to grow in the presence of certain antibiotics. The transconjugants resembled GA121 and differed from strain GAE10 in being (*a*) resistant to nalidixic acid (25 μg ml^{-1}) and streptomycin (200 μg ml^{-1}), (*b*) unable to

utilize xylose, galactose, raffinose, lactose, melibiose and arabinose, (*c*) inhibited by valine and (*d*) susceptible to lysis and lysogenization by phage P1. The transconjugants resembled GAE10, and differed from GA121, in growing on sucrose and in carrying one or more R factors which conferred resistance to tetracycline (25 μg ml^{-1}), kanamycin (30 μg ml^{-1}), neomycin (30 μg ml^{-1}), ampicillin (25 μg ml^{-1}) and chloramphenicol (25 μg ml^{-1}).

Tests for linkage between the resistance genes and the gene for sucrose utilization

The R factor in GAE10 did not transfer the gene for sucrose utilization on other occasions. It therefore seemed that a gene for sucrose utilization may have become associated with an R factor and been transferred with it to strain GA121. Tests were made to see whether loss of the R factor from a GAE10 × GA121 hybrid resulted in loss of the ability to use sucrose. Strain GA501 bacteria were treated with sodium dodecyl sulphate (SDS) as described by Salisbury *et al.* (1972). The treated suspension was plated on non-selective medium and 550 colonies were picked and tested; 211 were unchanged, 103 had lost only tetracycline resistance, 3 had lost tetracycline and chloramphenicol resistance, 113 had lost tetracycline, chloramphenicol and kanamycin resistance and 120 had lost resistance to the three antibiotics and also the ability to use sucrose. Untreated hybrid bacteria did not lose the characters. When GAE10 was treated with SDS in the same way, about 40% of the bacteria lost all the resistance characters but none lost the ability to use sucrose. The evidence suggests that a gene for sucrose utilization had become associated with the R factor. To test whether the sucrose character and the R factor were transmissible together in conjugation, strains GAE10 and GA501 were incubated with appropriate recipient bacteria of K12 and *Salmonella typhimurium* LT2 under conditions which allowed transfer of the R factor by conjugation, and the mixtures of bacteria were then plated on media which selected for recipients which had received resistance determinants. The transconjugants were tested by replica-plating, after restreaking, to see if they were able to use sucrose. In the first conjugations involving GA501 as donor, it was found that about 5% of transconjugants were *sac*$^+$; in later experiments, the resistance determinants were still transferable but the *sac*$^+$ gene was not transferable. In similar experiments with strain GAE10, the R factor was transferred to K12 but the *sac*$^+$ gene was not. Tests were done to see whether the sucrose-utilization gene and the resistance genes were cotransducible from GA501; selection was made separately for transfer of resistance genes and of *sac*$^+$. Over 1000 transductants which had received one or more resistance genes were tested but none were *sac*$^+$. Nevertheless, the gene for sucrose utilization was separately transducible, although at a lower frequency (1 in 10^7 phage) than the resistance genes (1 in 10^5 to 10^6 phage).

Strain K12 *bacteria did not mutate to use sucrose*

In the experiments described in this report, and in many other experiments, control bacteria of strains of K12 plated on sucrose minimal medium never mutated to use sucrose. In the experiment which achieved the transfer of *sac*$^+$ from GAE10, the numbers of K12(*sac*$^+$) bacteria which were obtained were very small and the possibility was considered that the *sac*$^+$ bacteria may have been rare mutants of K12 which had occurred, by chance, in the mixed cultures and not in the controls. The possibility of mutation to use sucrose was considered seriously because Coetzee (1962) showed that some groups of *Proteus* characteristically mutate to become sucrose-utilizing. A large number and variety of tests were therefore made to see whether K12 bacteria mutated to use sucrose, including all the tests described by Coetzee (1962). The tests included incubation with deoxycholate and incubation for long periods in peptone broth containing sucrose. Mutants able to use sucrose were not obtained.

Other workers have indicated that K12 bacteria do not utilize sucrose, and K12 bacteria

Table 2. *Cotransduction frequencies in the sac+ region*

Donor	Recipient	Allele generating selected phenotype	Transductants per 10^7 phage	No. scored	Unselected donor allele scored	Cotransduction frequency (%)
GA122	AB265 *his*	*sac+*	3	136	*his+*	0
		his+	45	98	*sac+*	0
GA122	AB347 *aroC*	*sac+*	1	134	*aro+*	62
		aro+	2	139	*sac+*	42
GA122	AB352 *purF*	*sac+*	20	43	*pur+*	5
		pur+	50	69	*sac+*	8
GA122	EM3000 *purF*	*sac+*	4	200	*pur+*	6
		pur+	25	160	*sac+*	17
GA515	AB347 *aroC*	*sac+*	15	84	*aro+*	59
		aro+	40	80	*sac+*	40
GA501	EM3000 *purF*	*sac+*	20	130	*pur+*	17
		pur+	20	78	*sac+*	9
GA501	GA301 *purF aroC*	*sac+*	2	150	*aro+*	30
					pur+	18
		aro+	2	160	*sac+*	66
					pur+	66
		pur+	10	122	*sac+*	9
					aro+	21
GA501	AB2547 *purF supN*	*sac+*	3	226	*pur+*	29
					sup+	30
		pur+	50	195	*sac+*	18
					sup+	0
AB1621	EM3003 *aroC dsdA*	*aro+*	10	180	*dsd+*	23
		dsd+	15	235	*aro+*	28

have been used as recipients in experiments demonstrating that some plasmids carry genes for sucrose utilization (Le Minor *et al.*, 1973; Smith & Parsell, 1975).

Mapping of the gene for sucrose utilization by conjugation

To map the *sac+* gene, three Hfr strains were made *sac+* by transduction from GA501. The Hfr(*sac+*) strains were used in time-of-transfer experiments with appropriate K12 recipients. The Hfr strains were AT997 (a derivative of KL16), AB2575 (of Hfr Hayes type) and AB312. Their *sac+* derivatives transferred gene *sac+* after 18, 55 and 65 min, respectively. The data indicated a map position for the gene in the min 45 to 50 region, clockwise to *his* at min 44.

Gene order was purF–aroC–(sac+)–supN

The *sac+* gene, after introduction into K12, was tested for cotransduction with genes *his*, *nalA*, *purF*, *aroC*, *dsdA* and *dapE* (shown in their order on the linkage map, from min 44 to min 53, with certain genes omitted). The donor strains were GA501 and derivatives of GA121 and AB1515 which had been transduced to *sac+* using GA501 as donor. Selection was made separately for *sac+* and for the other wild-type gene under test, and transductants were tested for the presence of the unselected donor allele. The wild-type alleles of all the genes were transduced separately with satisfactory frequencies, except that the *dsd+* gene was not transducible: the special problem of gene *dsd* is described below. The *sac+* gene was cotransducible with only two of the genes, *purF* and *aroC*, with frequencies of about 20% and 60%, respectively, indicating that *sac+* was close to *aroC* (Table 2).

The order of *sac+* relative to *purF* and *aroC* was found by transduction. Phage grown on GA501 was used to transduce GA301 *purF aroC*. Selection was made for *purF+*, *aroC+* and *sac+* in all combinations. Table 3 shows that the gene order was *purF aroC* (*sac+*).

To check whether GA501 was typical, strains GA502 to 509 were also used as donors in

Table 3. *Transductions to establish the relative position of gene sac+*

The data show the distribution of donor alleles amongst transductants of each selected class. The numbers in parentheses are the frequencies of transductants per 10^7 phage. The *sac+* gene is shown in its probable position in relation to the known sequence *purF–aroC–supN*. s, Selected allele; +, unselected donor allele received by transductants.

Transduction: donor GA501 *purF+ aroC+* (*sac+*), recipient GA301 *purF aroC*

No. scored for each selection	Donor alleles present			No. of transductants of each kind
	purF+	*aroC+*	*sac+*	
122	s			96
(10)	s	+		16
	s		+	0
	s	+	+	10
160		s		15
(1·5)	+	s		40
		s	+	40
	+	s	+	65
150			s	105
(2)	+		s	0
		+	s	18
	+	+	s	27
32	s	s		10
(10)	s	s	+	22
28		s	s	16
(4)	+	s	s	12
5	s		s	0
(2)	s	+	s	5

Transduction: donor GA501 *purF+* (*sac+*) *supN+*, recipient AB2547 *purF supN ilv*

No. scored for each selection	Donor alleles present			No. of transductants of each kind
	purF+	*sac+*	*supN+*	
195	s			160
(50)	s	+		35
	s		+	0
	s	+	+	0
226		s		98
(2.5)	+	s		58
		s	+	62
	+	s	+	8
51	s	s		48
(1·5)	s	s	+	3

transduction with GA301. Selection was made for transduction of *sac+*; each experiment gave at least one *sac+* transductant per 10^7 phage. About 80 *sac+* transductants were analysed from each transduction; the frequencies of cotransduction of *purF* and *aroC* were similar to those given by GA501. Summing the data for donors GA502 to 509, of 619 *sac+* transductants, 93 were *purF+* and 229 were *aroC+*.

The position of *sac+* relative to *supN* (Eggertsson & Adelberg, 1965) was also determined. The donor was GA501 and the recipient was AB2547 *purF supN ilv*. Selection was made for *sac+* and *purF+*, separately and together, and the transductants were tested for unselected markers (Table 3). In the recipient the *ilv* allele was suppressed by *supN* and the bacteria did not require isoleucine and valine, except when they received *supN+* by transduction. Table 3 shows that the gene order was *purF* (*sac+*) *supN*. Taken with the order *purF aroC* (*sac+*) established above, the data show that the order was *purF aroC* (*sac+*) *supN*.

Introduction of the sac⁺ region caused K12 *bacteria to be unable to use* D-*serine*

For much of the project it was thought that the *sac*⁺ region had replaced one or more of the *dsd* genes. Early in the project K12(*sac*⁺) bacteria were treated with mutagens and plated on media selecting for bacteria able to use D-serine, to see whether the *dsd* genes were present but inactive. Mutants able to use D-serine were not obtained. A later experiment showed that such mutants occurred with low frequency. Some were unable to use sucrose. This evidence suggests that the *sac*⁺ region did not displace the *dsd* genes.

Judging from the evidence of the preceding section, gene *sac*⁺ had taken a position close to genes *dsdAOC* in the otherwise barren region 1·5 min long between *aroC* and *supN* (McFall, 1967*a*, *b*; Bachmann *et al.*, 1976). Gene *dsdA* codes for D-serine deaminase (D-serine dehydratase; EC 4.2.1.14) which enables K12 to use D-serine as a carbon and energy source, and genes *dsdO* and *dsdC* are regulatory genes (McFall, 1964*a*,*b*, 1975; Bloom *et al.*, 1975). To determine the relative order of the *sac*⁺ and *dsd* genes, phage grown on GA501 was used to transduce EM3003 *purF aroC dsdA7* and selection was made separately for *aroC*⁺, *sac*⁺ and *dsdA*⁺ transductants; *aroC*⁺ and *sac*⁺ transductants were obtained but *dsd*⁺ transductants were not. The frequencies of *aroC*⁺ and *sac*⁺ transductants validated the transduction procedure. The lack of *dsd*⁺ transductants was caused by donor GA501 which proved unable to use D-serine. The K12 strain from which it derived was able to use D-serine. To check that the *dsd*⁺ character was being scored correctly, *dsd*⁺ and *dsd* strains from Professor Elizabeth McFall were compared with the strains which had already been used. The validity of the scoring procedure was confirmed. Strain EM3003 was transducible to *dsd*⁺ by other *dsd*⁺ donors, confirming that the difficulty was caused by GA501.

As a preliminary to finding the order of genes *sac*⁺ and *dsd*⁺ by transduction, it was desirable to make GA501 *dsd*⁺ as well as *sac*⁺ and this was attempted by transducing gene *dsd*⁺ from AB1621 into GA501. Selection was made for transductants able to use D-serine; the *dsd*⁺ transductants were then tested for ability to use sucrose. None were able to use sucrose, showing that introduction of *dsd*⁺ caused loss of the ability to use sucrose. The converse was also true; many *sac*⁺ transductants of K12 were available from transductions involving GA501 as donor, and all were unable to use D-serine. The wild donor of the *sac*⁺ gene was also unable to use D-serine, as were the hybrids GA502 to 509.

In further transduction experiments involving GA501 as donor and K12 *dsd*⁺ strains as recipients, 500 *sac*⁺ transductants were tested, and all were unable to use D-serine. When the direction of transduction was reversed, and selection was made for transductants able to use D-serine, not one of 185 transductants was *sac*⁺.

In two experiments the frequency of cotransduction of *aroC*⁺ and *dsd*⁺ was measured. The recipient was EM3003 *aroC dsdA7*. One donor was a *dsd*⁺ transductant of GA501 and was unable to use sucrose. Of 675 *dsd*⁺ transductants, 30% were *aroC*⁺, and of 330 *aroC*⁺ transductants, 23% were *dsd*⁺. None were *sac*⁺. The results when the donor was AB1621 are in Table 2. McFall (1967*a*) reported cotransduction frequencies of 49% and 25% for *aroC* and *dsd*.

Phage grown on GA501 was also used to transduce strain EM1600 which is constitutive for the deaminase because of a mutation in the regulatory gene *dsdO* (McFall, 1973). Over 1000 *sac*⁺ transductants were tested for their *dsd* character; none were able to use D-serine.

Independent evidence that the locus occupied by gene sac⁺ in K12 *was the locus it occupied in wild strain* GAE10

It was possible that the position taken by gene *sac*⁺ in the K12 chromosome had no relation to the position it occupied in wild strain GAE10. For example *sac*⁺ may have entered a region of the K12 chromosome which had affinity for the R factor. The *sac*⁺ region was therefore transferred to K12 by a different method, using Hfr (or R′) derivatives

of GAE10(R+). The Hfr (or R′) strains were isolated from a *trp met* mutant of GAE10(R+) by the method of Taylor & Adelberg (1960). One of the putative Hfr or R′*sac*+ strains was then tested to see whether the *sac*+ gene mapped at the same position as in the GA501 to 509 hybrids. In standard time-of-transfer experiments with K12 strains as recipients, it was found to have the gene-transfer properties of an Hfr strain, except that transfer was infrequent, about three *sac*+ colonies being obtained per 1000 donor bacteria. Gene *sac*+ was transferred by the donor after the same time interval as genes *purF*+ and *aroC*+, and the *sac*+ transconjugants had lost the ability to use D-serine as carbon and energy source. The donor was stable; it was tested after one year and had retained the ability to transfer the *sac*+ gene.

The transconjugant colonies from the experiment in which the GAE10 Hfr strains had been detected were also tested. They were all *sac*+, and unable to use D-serine, but otherwise retained the characters of AB1621, and were sensitive to phage P1. One was tested, as donor, in transduction experiments. The *sac*+ gene was cotransducible with *aroC*, and the *sac*+ transductants did not use D-serine.

D-*Serine deaminase activity was absent from* K12(*sac*+) *bacteria*

It seemed likely that the *sac*+ region in some way interfered with the formation or action of the enzyme D-serine deaminase (EC 4.2.1.14). The fact that K12(*sac*+) bacteria were inhibited by D-serine was evidence that the *sac*+ region did not affect uptake of D-serine. Appropriate K12 strains were assayed by the method of Pardee & Prestidge (1955) as modified by McFall (1964*a*). In one experiment, typical of several, bacteria were grown in complete medium to a density of 2×10^8 bacteria ml^{-1} and resuspended in minimal medium without glucose but containing D-serine (150 μg ml^{-1}); samples were taken at the time of adding D-serine (time 0) and at 30 min intervals for 2 h, and assayed for D-serine deaminase. The constitutive mutant EM1600 had a specific activity of about 16 units from time 0 onwards. Strain GA121 showed inducible activity, rising from less than 0·5 units after 1 h to about 17 units after 1·5 h. Strain GA501(*sac*+) and a *sac*+ transductant of EM1600 showed no activity at any time, and the deaminase-less mutant EM3003 was also devoid of activity. The activity shown by the constitutive and inducible bacteria was of the order reported by McFall (1964*a*).

The dsd+ *genes were not expressed in* K12 *dsd*+(F′*sac*+) *partial diploids*

In considering how *sac*+ bacteria failed to use D-serine there seemed to be three possibilities: (*a*) the *dsd*+ region may have been replaced by the *sac*+ region; (*b*) the *sac*+ region may have become integrated into the *dsd*+ genes and inactivated them; (*c*) the *dsd*+ genes may have remained intact but the *sac*+ region may have produced a cytoplasmic product which blocked expression of the *dsd* genes. To test the third possibility, partial diploids were made by isolating F′*sac*+ plasmids and inserting them into K12 *dsd*+ bacteria.

F′ plasmids carrying the *sac*+ region were selected from *sac*+ transductants of Hfr AB312, which transferred *sac*+ after about 70 min. The bacteria were treated with ultraviolet radiation to kill 90% and were used as donors in conjugation with JC5466 *trp his recA* as recipient. A *recA* recipient was used to prevent entry of *sac*+ into the recipient's chromosome; *recA*+ was 5 min distal to *sac*+ in the donor. A sample of conjugating bacteria was taken after 5 min and selection was made for *sac*+ recipients; the donor was counterselected by omitting threonine and leucine from the medium. Colonies occurred with a frequency of 1 per 10^4 donor bacteria. Ten colonies, denoted GA601 to 610, were tested to see if they were donors of *sac*+, with AB1621 serving as recipient. All behaved as if they contained F′ plasmids, transferring the *sac*+ gene with high frequency within 5 min. The F′ plasmids carried *purF*+ and *aroC*+, and four carried longer regions.

An F′ carrying the shortest chromosomal insert was used for making partial diploids.

Donor GA601 *trp his recA* (F'*purF*$^+$*aroC*$^+$*sac*$^+$) was incubated with AB1621, and selection was then made for *sac*$^+$ bacteria which did not require tryptophan and histidine. Many were obtained: they were assumed to be the required partial diploids in which the *dsd*$^+$ and *sac*$^+$ regions were present on different replicons in the same bacteria. Sixty were purified and tested for ability to use D-serine and sucrose as carbon and energy sources. None were able to use D-serine, indicating that the *sac*$^+$ region acted through the cytoplasm to make K12 bacteria unable to use D-serine. To test whether *sac*$^+$ would block expression of the genes in a mutant which synthesized the deaminase constitutively, partial diploids were made in conjugation between GA601 *recA his trp* (F'*purF*$^+$*aroC*$^+$*sac*$^+$) and EM1600 *dsdA*$^+$ *dsdO dsdC*$^+$, the recipient being constitutive for D-serine deaminase (McFall, 1973). Selection was made for *sac*$^+$ bacteria which did not require histidine and tryptophan. Ninety-five partial diploids were restreaked and tested. All were able to use sucrose and unable to use D-serine. To check that the *dsd*$^+$ region was still present, even though it was not achieving phenotypic expression, one of the partial diploids was used as donor in transduction with a *dsdA* mutant as recipient. The donor was EM1600 *dsdO*^{c} (F'*sac*$^+$) and the recipient was EM3003 *dsdA7*. Selection was made for transductants able to use D-serine; they were obtained with normal frequency. One of the *dsd*$^+$ transductants was tested for the presence of D-serine deaminase; the enzyme was present and constitutive, confirming that the genes *dsdO*^{c} and *dsdA* were present but not expressed in the partial diploids.

Strain K12(*sac*$^+$) *bacteria showed frequent mutation to resistance to* D-*serine and* D-*cycloserine*

In *E. coli* B and K12, D-serine is detoxified by D-serine deaminase, an inducible enzyme (Pardee & Prestidge, 1955; McFall, 1964*b*). When K12 is exposed to D-serine, growth is at first strongly inhibited, but once sufficient deaminase has formed to detoxify D-serine, the amino acid serves as a source of carbon and energy (McFall, 1964*a*). Curtiss *et al.* (1965) isolated mutants of K12 which were resistant to D-cycloserine and unable to take up D-cycloserine, D-serine and D-alanine. Robbins & Oxender (1973) and Cosloy (1973) obtained evidence for a permease, called 'dag' permease, which transports D-serine, D-alanine and glycine.

Mutants which are defective in D-serine deaminase do not utilize D-serine as a carbon and energy source and are inhibited when plated on glucose minimal medium containing D-serine (500 mg l^{-1}). We found that when K12 bacteria were made *sac*$^+$ they were similarly inhibited by D-serine but they gave frequent mutants which were resistant to D-serine. Possibly the resistant mutants were impermeable to D-serine. They did not use D-serine as a source of carbon and energy. Frequent mutation to D-serine resistance was shown only by K12(*sac*$^+$) strains. They gave about one D-serine resistant colony per 10^5 bacteria plated, whereas strain EM3003 *dsdA7* gave resistant colonies only when treated with a mutagen. Other K12 strains were not tested for mutation to D-serine resistance, because they possessed the *dsd*$^+$ genes and were able to detoxify and utilize D-serine. Transduction tests, at first sight, suggested that the resistance mutation was cotransducible with *sac*$^+$, but the evidence was invalid because the *sac*$^+$ transductants mutated to D-serine resistance sufficiently frequently to score as resistant when the colonies were replicated.

The K12 *dsd*$^+$(F'*sac*$^+$) partial diploids described in the preceding section also showed frequent mutation to D-serine resistance.

Cosloy (1973) described a mutation which greatly impaired the ability of bacteria to accumulate D-serine, D-alanine and glycine, and conferred resistance to D-cycloserine at 30 mg l^{-1}. This confirmed the conclusion of Kessel & Lubin (1965) and Wargel *et al.* (1971) that D-serine was transported by a system responsible for uptake of D-cycloserine. The gene was given the symbol *dag*. Cosloy (1973) and Robbins & Oxender (1973) mapped gene *dag* at min 94.

The D-serine resistant mutants arising in K12(*sac*$^+$) strains were resistant to D-cycloserine. D-Cycloserine at 20 mg l^{-1} completely inhibited growth of K12 strains, whether *sac*$^+$ or not, and resistant mutants were not observed. At 10 mg l^{-1}, K12(*sac*$^+$) derivatives gave resistant mutants, and other K12 strains did not; the frequency was about 1 per 10^6 bacteria plated. Once isolated, the mutants were resistant to 20 mg l^{-1}. They were also resistant to D-serine (500 μg ml^{-1}). Strain EM3003 *dsdA7* was inhibited by cycloserine and did not mutate to resistance at the high frequency shown by K12(*sac*$^+$). The evidence suggests that the mutations to D-serine resistance in K12(*sac*$^+$) may have been in gene *dag*.

Strain K12(*sac*$^+$) *bacteria mutated to use raffinose*

Strain K12 bacteria do not use raffinose and do not mutate to use it. Strain K12(*sac*$^+$) bacteria mutated to use raffinose with a frequency of about 1 per 10^5 bacteria plated. The mutations were independent of the mutations to D-serine resistance. They were not induced by the *sac*$^+$ region and are not further considered here. Smith & Parsell (1975) reported that K12 bacteria which received plasmid-borne genes for sucrose utilization mutated to use raffinose.

Gene sac$^+$ *was cotransducible with gene ptsI*$^+$ *and required ptsI*$^+$ *for expression*

Clockwise on the map, within 0·1 min of *supN*, are genes *ptsH* and *ptsI* of the phosphotransferase system for transport of carbohydrates (Roseman, 1969, 1972; Epstein *et al.*, 1970). Since it seemed that *sac*$^+$ might confer permeability to sucrose, the degree of linkage between *sac*$^+$ and the *pts* genes was of interest because of the possibility of a functional relationship. Authentic *ptsH* and *ptsI* mutants were provided by Professor W. Epstein. Mutant FF8040 *ptsI* was chosen for transduction experiments because it was the most stable in our experiments. Ability and inability to grow on mannitol and sorbitol were taken as the criteria for distinguishing *ptsI*$^+$ and *ptsI* transductants. Using GA501 as donor and FF8040 *ptsI* as recipient, selection was made separately for transductants able to grow on sucrose, sorbitol and mannitol. The *ptsI* mutant did not revert on control plates. Sorbitol-utilizing and mannitol-utilizing transductants were all able to use both hexitols and were scored as *ptsI*$^+$. Of 881 *ptsI*$^+$ transductants, 12% were *sac*$^+$. About 2 *ptsI*$^+$ transductants were obtained per 10^6 phage, but selection for *sac*$^+$ gave only about 3 transductants per 10^8 phage, and all were *pts*$^+$. This suggested that *sac*$^+$ required *ptsI*$^+$ for expression.

To test whether *sac*$^+$ was expressed in *pts* mutants, new *pts* mutants of a *sac*$^+$ transductant of EM3000 were isolated on dehydrocholic acid/neutral red agar (Morse & Alire, 1958) containing sorbitol and mannitol. Eight white mutant colonies were isolated; they were mannitol, sorbitol and sucrose negative. Six were stable. To test whether they were genotypically *sac*$^+$, one was used as recipient in transduction with AB1621 as donor. Selection was made for mannitol-positive and sorbitol-positive transductants. Of 301 transductants tested, all grew on mannitol, sorbitol and sucrose, confirming that the recipient was genotypically *sac*$^+$. Transductants which were *pts*$^+$ but unable to use sucrose were expected but not obtained. Taken together, these data show that *sac*$^+$ depended upon genes of the phosphotransferase system for expression. The cotransduction frequency of 11% was in agreement with the interval of about 1·3 min between *dsd* and *ptsI* shown on the linkage map.

DISCUSSION

The transfer of chromosomal genes for sucrose utilization has not been reported before, but some plasmids transmit the ability to use sucrose (Le Minor *et al.*, 1973; Smith & Parsell, 1975; Wohlhieter *et al.*, 1975). The symbol *sac*$^+$ (Le Minor *et al.*, 1973) seems to have priority, and is the symbol used for *Bacillus subtilis* (Lepesant *et al.*, 1972). The term '*sac*$^+$ region' will be used when effects may be due not to *sac*$^+$ but to accompanying DNA.

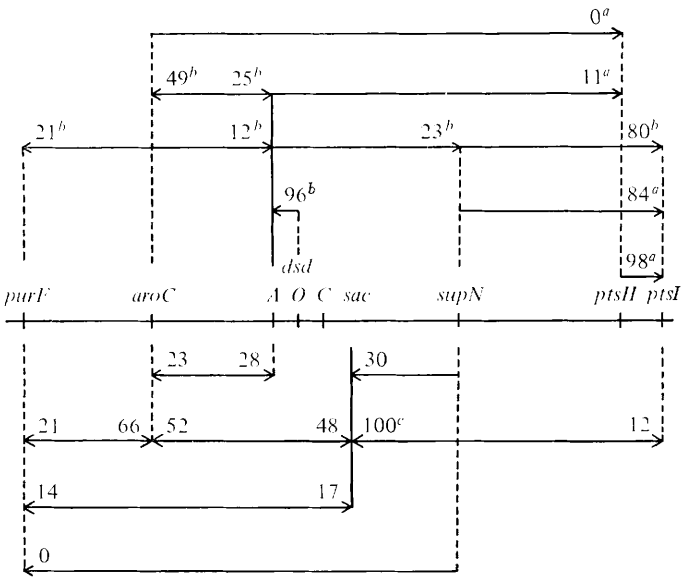

Fig. 1. Map showing the relative position of *sac*+ in *E. coli* K12. Cotransduction frequencies are given as the number of recombinants inheriting the unselected marker, expressed as a percentage of the number of transductants tested. For each pair of genes, the average cotransduction frequency is shown near the arrowhead pointing to the selected marker. *a*, Data of Epstein *et al.* (1970). *b*, Data of McFall (1967*a*). *c*, Gene *sac*+ is not expressed in *ptsI* mutants.

The evidence of cotransduction points to *sac*+ having taken a chromosomal position in K12. The data are summarized in Fig. 1. The fact that it took, at least approximately, the same position by two methods of transfer indicates that the region has affinity for the *dsd* region and that the methods of transfer did not determine the position taken. The way in which the *sac*+ region first entered the K12 chromosome is not known. It may have entered by homologous pairing and even numbers of crossovers or it may have behaved as a transposable element (see below). Since K12 need not be devoid of all *sac* DNA, introduction of only a part of a gene or operon may have enabled K12 to use sucrose. Reversion of *sac*+ bacteria to use D-serine indicated that the D-serine genes were not replaced. Possibly *sac*+ may have replaced a part of the *dsd*+ genes, or become inserted into them. Closely related may be the question of how *sac*+ prevented utilization of D-serine by partial diploids. One possible explanation may be that a protein from the *sac*+ region interacted with *dsd* nucleic acid, e.g. to block transcription. Another possible explanation may be interaction of gene products; for example, protein subunits may have had mutual affinity and aggregated in such a way that an active *dsd* protein was not formed. It seems more than coincidence that *sac*+ mapped close to *dsd* and interacted with it. Mutation of K12(*sac*+) to use D-serine permits genetical analysis of the interaction. The test of significance of the interaction, in relation to the aims of the project, is whether *sac*+ and *dsd*+ genes from other wild strains interact.

Strain K12(*sac*+) bacteria showed increased mutation to resistance to D-serine and D-cycloserine compared with a K12 *dsd* mutant. The mutations may have been in gene *dag*. One possible explanation is that ordinary K12 bacteria may have two systems which transport both substances, and one system may have been rendered inactive on introduction of the *sac*+ region, thereby allowing mutations which inactivate the second system to be expressed as resistance mutations. Another possible explanation is that an insertion sequence or transposable element or mutator gene may have been introduced or generated.

The ability of K12(*sac*$^+$) to use sucrose was dependent upon at least one gene of the phosphotransferase (PT) system. Sucrose is not reported to be transported by the PT system. Wang *et al.* (1969, 1970) observed that some *ptsI* mutants did not grow on compounds such as lactose which are not transported by a PT mechanism. Saier & Stiles (1975) suggest that a regulatory protein inhibits the transport of non-PT sugars, except when the protein is inactivated under appropriate conditions by a phosphorylation process involving the *ptsI* product, Enzyme I. The problem is discussed by Kornberg & Jones-Mortimer (1977).

When strain GA501 was first isolated, gene *sac*$^+$ gave evidence of being plasmid-borne. This raises the question of whether *sac*$^+$ may have acted as a transposable element (Hedges & Jacob, 1974; Cohen, 1976). A more general question is whether characters which are present in only a proportion of wild strains may be due to phage or other transposable elements. Transposability is now recognized to be of greater significance than when the present experiments were done. With regard to phage, tests were made to see whether supernatants of *sac*$^+$ bacteria were able to transfer the *sac*$^+$ gene without the addition of phage P1. The tests did not give positive results. With regard to the possibility of another kind of transposable element, transfer of the *sac*$^+$ region was first achieved in only one of many experiments with several *sac*$^+$ strains containing R factors. The evidence from conjugation experiments was that *sac*$^+$ took one position on the K12 chromosome and remained in that position. Translocation, if it occurred at all, seems not to have been a frequent property of the region under the conditions of the experiments, unless the effect on *dag* was due to translocation. The possibility remains that *sac*$^+$ may insert preferentially into the *dsd* region. Investigations concerned with this problem will be reported elsewhere.

The way in which the relationship between *sac*$^+$ and *dsd*$^+$ was discovered in this investigation illustrates the value of a detailed linkage map in the study of natural variation. The availability of simple and efficient methods of gene transfer, and of extensive and discontinuous variation in biochemical characters, may make *E. coli* a favourable subject for experiments in ecological genetics.

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