Genes for L-Sorbose Utilization in *Escherichia coli*

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Amongst forty wild strains of *Escherichia coli*, nine used L-sorbose as a source of carbon and energy and two mutated to use it. Laboratory strains K12, B and C were L-sorbose-negative. Genes for L-sorbose utilization (*sor*⁺) were transferred to K12 from six wild strains; genes conferring the mutable phenotype were also transferred. All were cotransducible with *metA* at 90 min on the linkage map. The most probable gene order was *met ace sor pgi mal*. Complementation tests identified two genes for L-sorbose utilization. Genetical evidence showed that the catabolite repressor protein of K12 exerted positive control over *sor*⁺ genes introduced into K12. The genes for phosphofructokinase (*pfkA*), the phosphocarrier protein (*ptsH*) and phosphotransferase enzyme I (*ptsI*) were required for utilization of L-sorbose.

The frequency of transduction of *sor*⁺ was low when selection was made for *sor*⁺, because L-sorbose partially inhibited the growth of both L-sorbose-negative strains and K12 (*sor*⁺) strains. Uridine, thymidine and sorbitol each annulled the inhibition of growth and increased the frequency of transduction of *sor*⁺.

INTRODUCTION

Alaeddinoglu & Charles (1979) confirmed the observation of Edwards & Ewing (1972) that about 50% of *Escherichia coli* strains, including K12, do not use sucrose. Transferred from a wild strain, the genes for sucrose utilization (*sac*⁺) were located at 50.5 min on the K12 linkage map. Hill (1980) and Hill & Charles (1980) showed that *sac*⁺ genes from twenty wild strains, and *sac* genes from wild strains that mutated to use sucrose, had the same location in K12, as judged by simple cotransduction tests. Only in occasional strains were genes for sucrose utilization carried by plasmids. Woodward (1980) and Woodward & Charles (1980*a, b*) set out to test whether genetical variation shown by other characters followed similar rules. Firstly, characters were transferred to K12 for ease of genetic analysis. The choice of characters was limited by the requirement for clear distinction between the different states of a character occurring in nature, and by the requirement that one state, usually the positive one, was easily selectable in transduction and conjugation experiments; it was also necessary that K12 be naturally negative for the character, to permit transfer to K12. Only characters specified by chromosomal genes were studied in detail, on the supposition that variation due to chromosomal genes might reflect less ephemeral selective effects in nature than variation due to the presence and absence of plasmids.

One character, L-sorbose utilization, gave results partly similar to those obtained for sucrose utilization (Woodward & Charles, 1980*a*). The characters of ribitol and D-arabitol utilization, on the one hand, and galactitol utilization on the other, exhibited a rather different situation in that their genes behaved as alternatives in the K12 chromosome (Woodward, 1980; Woodward & Charles, 1980*b*). The results obtained for the L-sorbose character are now presented. Genes specifically concerned with L-sorbose utilization are denoted *sor*.

METHODS

The methods used followed Alaeddinoglu & Charles (1979), except when stated otherwise.

Media. Glucose tetrazolium indicator medium was as described by Epstein *et al.* (1970).

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Table 1. *Escherichia coli* strains

Strain	Genotype	Reference
AB468	F ⁻ <i>proA2 lacY galK2 his-4 xyl mtl thi-1 purD</i>	Stouthamer <i>et al.</i> (1965)
AB1621	F ⁻ <i>ara lac tsx gal rpsL xyl mtl glpD thiA</i>	Adelberg <i>et al.</i> (1965)
AB2569	F ⁻ <i>proA2 lacY galK2 his-4 xyl mtl thi-1 argE metA</i>	Eggertsson & Adelberg (1965)
FF8020	F ⁻ <i>proC ptsH rpsL</i>	Epstein <i>et al.</i> (1970)
FF8040	F ⁻ <i>proC ptsI rpsL</i>	Epstein <i>et al.</i> (1970)
JC1553	F ⁻ <i>leu his recA rpsL malA argG metB</i>	Clark & Marguiles (1965)
MLM161	F ⁻ <i>his rpsL mal mtl ilv metB glpK argH</i>	McConville & Charles (1979)
PC0132	F ⁻ <i>lacY gal-3 rpsL malA xyl mtl thi-1 purH47</i>	Stouthamer <i>et al.</i> (1965)
WA802	F ⁻ <i>lacY galK2 galT22 hsdR2 metB</i>	Wood (1966)
WA803	F ⁻ <i>lacY galK2 galT22 hsdS3 metB</i>	Wood (1966)
2K	F ⁻ <i>lacY hsdS918 rpsL thi-1 serB</i>	Colson <i>et al.</i> (1965)
5K	F ⁻ <i>thr-1 leu-6 lacY tonA21 hsdR514 rpsL thi-1</i>	Colson <i>et al.</i> (1965)
AB347	VHfr <i>thr-1 leu-6 ara-2 lacZ4 aroC4 rpsL thi-1</i>	Huang & Pittard (1967)
AB2575	Hfr <i>tsx ilv thiA</i>	Pittard <i>et al.</i> (1963)
AM1	Hfr <i>tonA22 relA1 pfkA1</i>	Morrissey & Fraenkel (1968)
AT997	Hfr <i>dapC relA thi-1</i>	Bukhari & Taylor (1971)
DF40	Hfr <i>tonA22 relA1 pgi-2</i>	Fraenkel & Levisohn (1967)
KG1673	Hfr <i>thi-1</i>	Kawasaki <i>et al.</i> (1968)
R4	Hfr <i>relA1 metB1</i>	Reeves (1959)
5333S	Hfr <i>rpsL crp</i>	Epstein & Kim (1971)
MW4	<i>sor</i> ⁺ transductant of strain R4, donor MW871	This work
MW5	<i>sac</i> ⁺ transductant of strain MW4, donor GA122 (Alaeddinoglu & Charles, 1979)	This work
MW284	<i>ace284</i> mutant of strain DF40 made by MNNG mutagenesis	This work
MW285	<i>sor</i> ⁺ transductant of strain MW284, donor MW871	This work
MW871-878	<i>sor</i> ⁺ transductants of strain AB1621, donor RK87	This work
MW951-953	<i>sor</i> ⁺ transductants of strain AB1621, donor RK95	This work
MW3016	<i>mal-3016</i> mutant of strain AB2569 made by MNNG mutagenesis	This work
MW3017	<i>sor</i> ⁺ transductant of strain MW3016, donor MW871	This work
MW5752	<i>sor</i> ⁺ transductant of strain AB2575, donor MW871	This work
MWSN50-75	<i>sor</i> mutants of strain MW5752 made by MNNG mutagenesis	This work
MWSN3050-75	<i>metA</i> ⁺ <i>sor</i> transductants of strain MW3017, donors were MWSN50-75 respectively	This work
RK1-120	Wild strains isolated from the River Kennet	This work

Lysogenization and transduction by P1clr100KM. These were as described by Goldberg *et al.* (1974). An improved method of lysogenization is described in the text.

Conjugation. For VHfr AB347, chromosome transfer was stopped by lysis from without, using phage T6; samples (0.1 ml) of plating mixture were diluted in 0.9 ml of a solution containing 0.1 M-MgSO₄, 0.02 M-CaCl₂, DL-tryptophan (4 mg l⁻¹) and 10⁸ infective phage T6. Lysis was at 37 °C for 15 min. Recipients were *tsx*.

Gene symbols and map distances. These follow Bachmann & Low (1980). By agreement with Dr Bachmann the genes for L-sorbose transport and L-sorbose utilization are given the symbols *sorT*⁺ and *sorA*⁺, respectively.

Strain designations and genotypes. These are shown in Table 1. Strain MW284 was made by mutagenesis of strain DF40; the new allele *ace-284* was similar to the *aceA* and *aceB* alleles (Brice & Kornberg, 1968) in blocking acetate utilization; cotransduction frequencies of 86% with *metA* and of 42% with *pgi* were close to those obtained by Vanderwinkel & de Vlieghere (1968). Strain MW3016 was made by mutagenesis of strain AB2569; the new allele *mal-3016* generated the *malB* phenotype described by Hofnung *et al.* (1974); cotransduction frequencies of 10% with *metA* and of 47% with *pgi* were close to those obtained for *malB* by Kadner & Liggins (1973).

RESULTS

Transfer to K12 of the ability to use L-sorbose

Escherichia coli K12 strains did not use L-sorbose as a carbon and energy source and did not mutate to use it. Of forty wild strains streaked on L-sorbose minimal medium, nine used L-sorbose and two gave L-sorbose-positive mutant colonies against a background of L-sorbose-

negative bacteria. Because no wild strains gave plaques with phage P1, attempts were made to lysogenize the L-sorbose-positive strains with phage P1*clr100KM* (Goldberg *et al.*, 1974), as a preliminary to transduction. Lysogens were selected by their kanamycin resistance, and lysogeny was confirmed by lysis at 42 °C and liberation of phage. Strains RK87 and RK95 were readily lysogenized, but other strains did not give lysogens. Induction at 42 °C gave lysates (10^9 phage ml⁻¹) which were used to transduce AB1621 (*xyl*), selection being made separately for transductants able to use L-sorbose and xylose. Selection for *xyl*⁺ served as a positive control measuring transducibility of a gene common to K12 and the donors. The *xyl*⁺ transductants occurred with a frequency of 1 per 4×10^6 phage, and were scoreable after 2 d. Strain RK87 gave eight Sor⁺ transductants from 10^9 phages, and RK95 gave three Sor⁺ transductants from 10^9 phages, all scoreable after 5 d. The Sor⁺ transductants were given the designations MW871 to MW878, and MW951 to MW953, respectively. Apart from being Sor⁺, transductants retained characters typical of AB1621. They differed from the wild donors in requiring 5 d, rather than 2 d, to give colonies of 2 mm diameter on L-sorbose minimal medium.

The possibility that K12 bacteria might mutate to use L-sorbose was checked carefully. A large number of varied tests all failed to give K12 mutants able to use L-sorbose. Tests included treatments with nine common chemical mutagens, UV irradiation, incubation for long periods in peptone broth containing L-sorbose, and incubation in L-sorbose medium containing bile salts, since Coetsee (1962) found that bile salts increased the permeability of *Proteus* to sucrose.

Tests were made to see whether ability to utilize L-sorbose was due to plasmids. Cells of *E. coli* K12 were incubated with Sor⁺ wild strains and with Sor⁺ mutants of the two mutable strains. The K12 recipients in different experiments were AB1621, 2K, 5K, WA802 and WA803, selection being made for Sor⁺ transconjugants resistant to streptomycin. The Sor⁺ character was not transferred in these tests, nor was it lost from wild strains on treatment with sodium lauryl sulphate (Tomoeda *et al.*, 1968) or acridine orange (Salisbury *et al.*, 1972) by methods which removed plasmids from K12 (Alaeddinoglu & Charles, 1979).

Gene order

Using hybrid MW871 as donor, Hfr strains KG1673, AB347, AT997 and AB2575 were transduced to Sor⁺. In time-of-transfer experiments, the Hfr strains transferred their *sor*⁺ regions after 20, 30, 75 and 100 min respectively, indicating a locus in the 89–91 min region near *malB* (Hofnung *et al.*, 1974). Tests were made for cotransduction of *sor*⁺ with the wild-type alleles of *argH*, *purH*, *purD*, *metA*, *ace-284*, *pgi* and *mal-3016*, which occur in that order on the linkage map, from 89 to 91 min, with certain genes omitted. In each transduction, selection was made separately for *sor*⁺ and wild-type allele of the relevant marker gene, with the exception that *pgi*⁺ was not selectable. Cotransduction of *pgi*⁺ was tested by streaking *sor*⁺ transductants on glucose tetrazolium indicator medium: *pgi*⁺ bacteria gave pale pink colonies and *pgi* bacteria gave dark red colonies (Fraenkel & Levison, 1967). In each cross, selection for *sor*⁺ gave less than one-twentieth the transductants given by the other selection (Table 2). The *sor*⁺ region was cotransduced with all the markers, most frequently with *pgi*⁺, *ace-284*⁺ and *mal-3016*⁺. MW285 (*ace-284 sor*⁺ *pgi*) was then used as donor in transduction with MW3016 (*metA mal-3016*), selections being made for *sor*⁺ and *metA*⁺. The results in Table 3 show that the gene order was probably *met ace sor*⁺ *pgi mal*. The other ten K12 (*sor*⁺) hybrids were tested in critical transductions (Table 4) and gave evidence for a constant locus for *sor*⁺.

Comparisons of cotransduction frequencies for the pairs of alleles *metA-ace-284*, *metA-pgi* and *metA-mal-3016* were made using as donors a K12 strain (MW284) and a K12 (*sor*⁺) hybrid (MW285). The recipient was MW3016 and selection was made in each case for *metA*⁺. Cotransduction frequencies (%) were 82.5 and 85, 18 and 21.6, and 8 and 9.8, respectively.

The transductants selected for *sor*⁺ in Table 3 were tested to see whether they might be unstable partial diploids. To determine their genotypes, the transductants had first been streaked on the selective medium, then patched on the same medium and replicated on to other media. One colony from each of the 527 streaks was restreaked four times on the same medium containing L-sorbose, methionine and uridine (see below). Forty-three transductants gave

Table 2. *Cotransduction frequencies in the sor⁺ region (donor strain MW871)*

Recipient	Allele generating selected phenotype	Transductants per 10 ⁸ phage	No. scored	Unselected donor allele scored	Contratransduction frequency (%)
MLM161	<i>sor</i> ⁺	10	210	<i>argH</i> ⁺	0
	<i>argH</i> ⁺	200	1321	<i>sor</i> ⁺	1.5
PCO132	<i>sor</i> ⁺	8	55	<i>purH</i> ⁺	9
	<i>purH</i> ⁺	200	164	<i>sor</i> ⁺	22
AB468	<i>sor</i> ⁺	10	79	<i>purD</i> ⁺	11
	<i>purD</i> ⁺	150	246	<i>sor</i> ⁺	28
AB2569	<i>sor</i> ⁺	20	90	<i>metA</i> ⁺	15
	<i>metA</i> ⁺	300	237	<i>sor</i> ⁺	34
MW284	<i>sor</i> ⁺	30	67	<i>ace</i> ⁺	24
	<i>ace</i> ⁺	100	124	<i>sor</i> ⁺	47
DF40	<i>sor</i> ⁺	10	100	<i>pgi</i> ⁺	77
MW3016	<i>mal</i> ⁺	40	230	<i>sor</i> ⁺	16
	<i>sor</i> ⁺	6	40	<i>mal</i> ⁺	34

Table 3. *Evidence from transduction for the position of sor⁺*

The donor was MW285 (*metA*⁺ *aceAB* *sor*⁺ *pgi* *malB*⁺) and the recipient was MW3016 (*metA* *aceAB*⁺ *pgi*⁺ *malB*); *sor*⁺ is shown in its most probable position relative to the known sequence of marker genes.

Unselected donor markers received when selection was for <i>sor</i> ⁺ *						Unselected donor markers received when selection was for <i>metA</i> ⁺ †					
<i>metA</i> ⁺	<i>ace</i>	<i>sor</i> ⁺	<i>pgi</i>	<i>mal</i> ⁺	No. in class	<i>metA</i> ⁺	<i>ace</i>	<i>sor</i> ⁺	<i>pgi</i>	<i>mal</i> ⁺	No. in class
—	—	S	—	—	156	S	+	—	—	—	264
—	—	S	+	+	123	S	—	—	—	—	87
—	—	S	+	—	106	S	+	+	+	—	71
+	+	S	—	—	28	S	+	+	—	—	65
—	+	S	—	—	26	S	+	+	+	+	43
+	+	S	+	—	17	S	—	+	—	—	6
—	+	S	+	+	16	S	+	—	—	+	6
+	+	S	+	+	13	S	—	+	—	+	2
—	+	S	+	—	13	S	+	+	—	+	2
+	—	S	+	+	9	S	—	+	+	+	1
—	—	S	—	+	6	S	+	—	+	+	1
+	—	S	+	—	4	S	—	+	+	—	1
—	+	S	—	+	4	S	—	—	+	—	0
+	—	S	—	—	4	S	—	—	+	+	0
+	—	S	—	+	1	S	—	—	—	+	0
+	+	S	—	+	1	S	+	—	+	—	0

S, selected marker; +, unselected donor marker received; —, unselected recipient marker retained.

* Transduction frequency 1 per 2×10^6 phage.

† Transduction frequency 1 per 10^6 phage.

microcolonies on each successive streak: microcolonies were tiny translucent colonies less than 0.5 mm in diameter, quite unlike the 2 mm opaque colonies which largely made up the streak. About one microcolony was present for every 200 large colonies on a streak. Transductants that gave microcolonies all belonged to the class of 156 in which only the *Sor*⁺ character from the donor had been received.

Transduction of the sor regions from six more wild strains

Several attempts were made to lysogenize strains which were not lysogenized by the standard method. Schell & Glover (1966*a, b, c*) described treatments which decreased the restriction

Table 4. Cotransduction of two markers with *sor*⁺ using 10 different K12 (*sor*⁺) hybrids as donors

Selection was for *sor*⁺. The recipient was MW3016 (*metA mal-3016*). Donor strains MW872 to 878, and MW951 to 953 contained *sor*⁺ regions from wild strains RK87 and RK95, respectively.

Donor	Transductants per 10 ⁸ phage	No. scored	Allele generating unselected phenotype	Cotransduction frequency (%)
MW872	10	97	<i>metA</i> ⁺	32
			<i>mal</i> ⁺	35
MW873	20	83	<i>metA</i> ⁺	24
			<i>mal</i> ⁺	33
MW874	8	79	<i>metA</i> ⁺	24
			<i>mal</i> ⁺	34
MW875	7	101	<i>metA</i> ⁺	14
			<i>mal</i> ⁺	36
MW876	6	71	<i>metA</i> ⁺	21
			<i>mal</i> ⁺	30
MW877	6	65	<i>metA</i> ⁺	20
			<i>mal</i> ⁺	26
MW878	10	93	<i>metA</i> ⁺	28
			<i>mal</i> ⁺	28
MW951	10	91	<i>metA</i> ⁺	19
			<i>mal</i> ⁺	31
MW952	15	93	<i>metA</i> ⁺	17
			<i>mal</i> ⁺	33
MW953	15	102	<i>metA</i> ⁺	19
			<i>mal</i> ⁺	32

Table 5. Isolation of *P1clr100KM* lysogens of *E. coli* wild strains

In method (i) 10⁹ bacteria were mixed with 2.5 × 10⁹ phage and plated as described by Goldberg *et al.* (1974); method (iii) is described in the text, and method (ii) was method (iii) without the period for expression of kanamycin resistance.

Strain	No. of presumptive lysogens obtained per 10 ⁹ bacteria		
	Method:		
	(i)	(ii)	(iii)
RK87	489	NT	NT
RK95	578	NT	NT
RK43	0	3*	417
RK53	10*	14*	952
RK79	6*	67*	2041
RK99	0	0	184
RK107	1*	0	104
RK119	5*	0	159
RK2	0	0	0
RK27	0	0	0

NT, Not tested.

* Did not lyse at 42 °C; probably spontaneous kanamycin-resistant mutants.

endonuclease activity of K12 and allowed phage from *E. coli* C to give more plaques on K12. Adaptation of their methods gave *P1clr100KM* lysogens of six more wild strains. Overnight cultures in complete medium were washed twice, incubated in 0.01 M-MgSO₄ solution (37 °C, 1 h), incubated in fresh MgSO₄ solution (49.5 °C, 1 h), resuspended in a solution of 0.01 M-MgSO₄ and 0.02 M CaCl₂ with the phage at a multiplicity of infection of 2.5 (30 °C, 30 min) and diluted with complete medium (5:1, v/v). Suspensions were then incubated at 30 °C for 40 min to allow phenotypic expression of kanamycin resistance and plated on complete medium supplemented with kanamycin (12.5 mg l⁻¹). Results for the three methods are shown in Table 5.

Table 6. *Testing of metA⁺ transductants given by six wild donors, for cotransduction of sor⁺ (or sor^m)* and mal-3016⁺*

Selection was made for *metA⁺*, using strain MW3016 (*metA mal-3016*) as recipient. Transductants from mutable donors were streaked and incubated on L-sorbose minimal medium for 14 d to test for ability to mutate to use L-sorbose.

Donor	Phage titre on Strain 5K	Transductants per 10 ⁷ phage	No. scored	Allele generating unselected phenotype	Cotransduction frequency (%)
RK43	5 × 10 ⁷	5	48	<i>sor⁺</i>	25
				<i>mal⁺</i>	15
RK79	7 × 10 ⁷	2	19	<i>sor⁺</i>	37
				<i>mal⁺</i>	16
RK99	1 × 10 ⁷	10	110	<i>sor⁺</i>	28
				<i>mal⁺</i>	8
RK107	1 × 10 ⁹	8	250	<i>sor⁺</i>	31
				<i>mal⁺</i>	17
RK53*	5 × 10 ⁸	2	129	<i>sor^m</i>	77*
				<i>mal⁺</i>	42
RK119*	2 × 10 ⁸	10	230	<i>sor^m</i>	22*
				<i>mal⁺</i>	17

* Denotes mutation to use L-sorbose, when streaked on L-sorbose medium.

Induction of the new lysogens gave suspensions containing at least 10⁷ phage ml⁻¹, which were used to transduce MW3016. Because strains RK53 and RK119 did not use L-sorbose, but mutated to use it, selection was made indirectly for *sor* by selecting for *metA⁺* in all crosses (Table 6). Selection was also made separately for *proA⁺* (6 min), to check that the *sor* region was not transferred indiscriminately with donor markers. Transductants were tested for cotransduction of *sor* and *mal⁺* genes. The *sor⁺* genes from L-sorbose-positive strains showed 25% to 31% cotransduction with *metA⁺*. Transductants receiving genes from L-sorbose-mutable donors were streaked on L-sorbose minimal medium and incubated for up to 14 d to see whether they gave L-sorbose-utilizing mutants; about 80% of the transductants derived from donor RK53, and 20% from RK119, gave mutants able to use L-sorbose. The *proA⁺* transductants neither grew, nor mutated to grow, on L-sorbose minimal medium.

Inhibitory effects of L-sorbose

Transduction of the *Sor⁺* character into K12 was unsatisfactory in two ways. Firstly, *Sor⁺* transductants of K12 grew less well than the nine *Sor⁺* wild strains when streaked on L-sorbose minimal medium; whereas wild strains gave uniform colonies of 2 mm diameter after 2 d on L-sorbose medium, the transductants required 5 d to give colonies which were not more than 2 mm and variable in diameter. Secondly, the average frequency of transduction of the *Sor⁺* character when selected directly was not more than 5% (and often much less) of the frequency of transduction of characters common to K12 and wild strains (such as *proA⁺*), regardless of whether transduction was from a wild strain to K12, or from a K12 (*sor⁺*) hybrid to a K12 strain. In fact, transduction of *sor⁺* was more frequent when it was cotransduced with a nearby marker such as *metA⁺*. These facts suggested that K12 (*sor⁺*) bacteria lacked genes or alleles necessary for uniform and vigorous growth, or initiation of growth, on L-sorbose. One possibility was that L-sorbose might be partially inhibitory towards K12, the inhibition not being overcome by making the bacteria *sor⁺* except in so far as the L-sorbose was detoxified by metabolism. When K12 and L-sorbose-negative wild strains were streaked on lactate minimal medium supplemented with L-sorbose, L-sorbose proved to be partially inhibitory. In the absence of L-sorbose, uniform colonies of 2 mm diameter were obtained in 3 d. In the presence of L-sorbose, colonies of each strain varied in size from 0.01 to 0.5 mm in diameter, except that RK31 and RK92 did not grow at all. The same degrees of inhibition occurred when acetate was the source of carbon and energy instead of lactate.

L-Sorbose did not decrease growth of colonies in the presence of glucose or fructose, suggesting that catabolite repression annulled the inhibition. Inhibition did not occur in complex media lacking sugars, suggesting that other metabolites also relieved the inhibition. In auxanographic tests, 212 biochemical substances were tested on AB1621 bacteria on lactate minimal medium containing L-sorbose. About 3000 bacteria were spread on each plate and the plates were examined after 2 d. Under these conditions the bacteria on control plates did not form visible colonies. Carbohydrates utilized by AB1621, namely glucose, fructose, trehalose, galactitol, rhamnose, ribose, mannose, L-fucose and sorbitol gave colonies of 2 mm diameter in their diffusion zones. Apart from sorbitol (90 mg l^{-1}), the sugars and sugar alcohols that allowed growth in the presence of L-sorbose only did so at concentrations above 270 mg l^{-1} , which supported significant growth in the absence of L-sorbose and lactate. Lactose, arabinose, galactose and other sugars not utilized by AB1621, did not give colonies. Two other substances were active, uridine and thymidine, and they gave colonies of 1 mm diameter. Uridine (50 mg l^{-1}) and thymidine (80 mg l^{-1}) in lactate medium containing L-sorbose each permitted vigorous growth of K12 strains and hybrids in the presence of L-sorbose. Uridine and thymidine also acted as sole sources of carbon and energy but did not support detectable growth (in the absence of L-sorbose) on plates at concentrations below 180 mg l^{-1} .

Thus the low frequency of transduction of *sor*⁺ probably resulted from the inhibitory effect of L-sorbose on recipient bacteria, before or after entry of donor DNA into the recipient's chromosome. This was confirmed in transduction experiments; when selection was made for *pro*⁺, *his*⁺, *met*⁺ and *arg*⁺, the number of transductants decreased 100-fold when L-sorbose was present in the lactate (or acetate) minimal medium. Uridine (50 mg l^{-1}), thymidine (80 mg l^{-1}) and sorbitol (90 mg l^{-1}), present with L-sorbose, each increased the frequency of all transductants from 1 per 5×10^7 phage to 1 per 2×10^6 . Growth of recipient bacteria in T2 broth supplemented with sorbitol (90 mg l^{-1}), before incubation with phage, increased the yield of *sor*⁺ transductants selected on L-sorbose minimal medium by 10-fold, but supplementation with uridine or thymidine had no effect. Unless stated otherwise, uridine (50 mg l^{-1}) was routinely added to L-sorbose minimal medium.

Two linked genes for L-sorbose utilization

To obtain evidence about the number of genes involved in L-sorbose utilization, *sor* mutants were isolated from K12 (*sor*⁺) hybrid Hfr MW5752, using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as mutagen. Of the 19 mutants obtained, 7 were stable and 12 mutated to use L-sorbose at frequencies of 1 per 10^8 to 1 per 10^{10} bacteria plated. Mutants defective in catabolism of a carbohydrate often show inhibition by that carbohydrate (Yarmolinsky *et al.*, 1959; Engleberg *et al.*, 1962; Solomon & Lin, 1972; Ferenci & Kornberg, 1973; Lengeler, 1977). The mutants were therefore tested for inhibition by L-sorbose. Washed bacteria were seeded in lactate minimal agar (without supplements) to give about 2×10^6 bacteria ml^{-1} , and a crystal of L-sorbose was applied to the solidified agar in each Petri dish. After 24 h, seven mutants showed zones devoid of growth, 5 cm in diameter, around the crystals; the other mutants gave zones of inhibition characteristic of K12 strains. The tests were repeated with uridine, thymidine and sorbitol in the medium; they annulled the inhibitions at concentrations of 200, 280 and 630 mg l^{-1} , respectively, giving uniform growth throughout the plates. It seemed likely that there were two genes for L-sorbose utilization, one concerned with transport (gene *sorT*) and one with enzymic conversion to sorbitol 6-phosphate (gene *sorA*). The L-sorbose-sensitive mutants were provisionally classified as *sorT*⁺*A*, and the rest as *sorTA*⁺ (or *sorTA*).

To test whether the mutations were in the *sor* region, P1kc lysates of the mutants were used to transduce MW3017 (*metA sor*⁺). Selection for *metA*⁺ gave about one transductant per 10^6 phage in each cross. One hundred transductants from each cross were tested for ability to use L-sorbose. All crosses showed cotransduction of *sor*, at frequencies between 28 and 41%. One *metA*⁺ *sor* transductant from each cross was tested; each showed the same sensitivity to L-sorbose as its donor parent, and the same frequency of reversion to *Sor*⁺.

To make complementation tests, F' plasmids carrying each *sor* region were obtained from conjugations with the form: Hfr MWSN50 (*ilv metB*⁺ *sor*) × recipient JC1553 (*leu his rpsL*

recA argG metB). Selection was made for early transfer of *metB*⁺, usually transferred late by this Hfr. Omitting isoleucine and valine from the plating medium, and adding streptomycin, gave selection against the donors. F primes were chosen that carried the chromosomal region from *metB*⁺ to the F factor (87–95 min) including the *sor* region at 89.7 min and *mal*⁺ at 91 min. The presence of *sor* mutations in twelve of the F' transconjugants was confirmed by their ability to mutate to Sor⁺. At this stage it was not possible to show that *sor* regions were present in F primes derived from stable *sor* mutants. Partial diploids carrying all possible pairs of *sor* mutations were made by transfer of the F' plasmids to the *metA*⁺ *sor* transductants described above, in conjugations of the form: donor JC1553 (*leu*/F'*sor malB*⁺) × recipient MWSN3050 (*sor mal*-3016), selection being made for *mal*⁺ transconjugants. Transconjugants were purified by streaking, and then tested by streaking on L-sorbose minimal medium and incubating at 37 °C for 60 h. Parental donor and recipient strains, tested as controls, did not utilize L-sorbose, nor did partial diploids carrying the same mutation on plasmid and chromosome. When either replicon carried *sor*⁺, it was expressed in the presence of any *sor* mutation. Complementation occurred when the *sor* region from any of the mutants MWSN50, MWSN56, MWSN57, MWSN58, MWSN61, MWSN72 and MWSN78 (comprising the *sorT*⁺*A* group strongly inhibited by L-sorbose) was present with the *sor* region from five *sorTA*⁺ mutants, namely MWSN51, MWSN52, MWSN70, MWSN75 and MWSN77. Diploids carrying other pairs of mutant regions gave streaks composed largely of minute colonies, but with varying numbers of larger and late developing colonies up to 1.5 mm in diameter; the frequency and nature of the larger colonies was not determined, but they probably contained *sor*⁺ regions generated by crossing over.

Sensitivity of two wild strains to L-sorbose

Amongst the 29 Sor⁻ wild strains, 27 showed the low degree of sensitivity to L-sorbose shown by K12 and K12 (*sorTA*⁺) mutants, but two showed high sensitivity typical of K12 (*sorT*⁺*A*) mutants. When seeded in lactate minimal agar and tested with crystals of L-sorbose, RK32 and RK91 gave zones of complete inhibition 6 cm in diameter, and required uridine at 120 and 140 mg l⁻¹ respectively to annul the inhibitions [compared with 200 mg l⁻¹ for K12 (*sorT*⁺*A*) mutants]. It seemed that RK32 and RK91 might be naturally-occurring *sorT*⁺*A* strains. To test whether sensitivity was due to genes in the *metA-malB* region, P1*clr100KM* lysogens of the wild strains were prepared and their lysates used to transduce MW3016 (*metA mal*-3016), with selection for *metA*⁺. Amongst 200 transductants from each donor, 20% and 6%, respectively, received *mal*⁺ but no transductants displayed the high sensitivity of the donors.

Genes for L-sorbose utilization

In *Klebsiella aerogenes*, transport of L-sorbose into the bacteria depends upon enzyme I of the phosphotransferase system and L-sorbose is phosphorylated to give L-sorbose 1-phosphate (Kelker *et al.*, 1972). Reduction to sorbitol 6-phosphate is followed by oxidation by D-sorbitol-6-phosphate dehydrogenase (EC 1.1.1.140) to D-fructose 6-phosphate. The latter is phosphorylated by phosphofructokinase (EC 2.7.1.11) to D-fructose 1,6-bisphosphate. Preliminary experiments showed that *sor*⁺ transductants were not obtained when using recipient strains carrying *ptsH* (FF8020), *ptsI* (FF8040), *pfkA* (AM1) or *crp* (5333S) mutations.

To confirm the requirement for *pts*⁺ gene function, *pts* mutants of MW871 were obtained, using MNNG and glucose tetrazolium indicator medium (Fraenkel & Levison, 1967). After overnight incubation on the indicator medium, 5 dark red colonies were obtained amongst many pink ones; they gave poor growth on glucose, fructose and maltose and none on sorbitol and L-sorbose. To obtain evidence that the mutants were *pts sor*⁺, they were used as recipients in transduction with strain MW5 (*sac*⁺ *pts*⁺) as donor. Genes *sac*⁺ (sucrose utilization) and *pts* are 11% cotransducible (Alaeddinoglu & Charles, 1979). Transductants able to use sorbitol were selected; they occurred with a frequency of 1 per 2 × 10⁶ phage. Of 200 tested, all utilized L-sorbose and 18 utilized sucrose.

To confirm that L-sorbose utilization was dependent upon *pfkA*⁺, the *pfkA* mutation was transduced into MW4 (*metB pfkA*⁺ *sor*⁺), using as donor AM1, and selecting for *metB*⁺, which is 30% cotransducible with *pfkA* (Vinopal *et al.*, 1975). Transductants occurred with a frequency of

1 per 2×10^6 phage. Of these, 50 were tested for cotransduction of *pfkA* by streaking on fructose (20 mM) minimal medium; 13 used neither fructose nor L-sorbose, and 37 used both. The presence of *sor*⁺ in the 13 Sor⁻ transductants was confirmed by using each as donor in conjugation with AB1621; selection was made for Sor⁺ transconjugants, and streptomycin was used to select against the donors.

Lengeler (1977) showed that *pfkA* mutants accumulate fructose 6-phosphate, the accumulation being related to inhibition of growth by fructose, sorbitol and mannitol. If L-sorbose catabolism proceeds *via* fructose 6-phosphate, then *pfkA sor*⁺ bacteria presented with L-sorbose may suffer inhibition of growth on lactate. This was shown to be so by streaking on lactate minimal medium and on lactate minimal medium supplemented with L-sorbose.

Confirmation of a requirement for *crp*⁺ was obtained by transducing the *crp* allele from 5333S into MW5 (*sac*⁺ *metB sor*⁺), and selecting for the streptomycin resistance allele *rpsL* (0.3 min from *crp*: Epstein & Kim, 1971) after incubating the phage-infected bacteria (30 °C, 24 h) in 10 ml complete broth, to allow phenotypic expression of resistance. About 30% of the *rpsL* transductants behaved as *crp* in being able to utilize glucose and fructose, but not lactose, galactose, arabinose, xylose, sucrose or L-sorbose.

Mutability and complementation tests with wild strains

The 29 Sor⁻ wild strains were tested for mutability to Sor⁺ using nine chemical mutagens including MNNG, but no mutant colonies were found.

Hill (1980) used F primes to demonstrate that sucrose-negative wild strains often possessed one or other of the *sac* genes in plus form. The *sorT*⁺*A*⁺, *sorT*⁺*A* and *sorTA*⁺ primes were used to test whether any Sor⁻ wild strains were T⁺ or A⁺. First it was shown that each of the 29 Sor⁻ wild strains was able to receive and express the *sor*⁺ genes. JC1553/F'*sor*⁺ served as donor in conjugations; selection was made on L-sorbose minimal medium containing uridine (50 mg l⁻¹, but 200 mg l⁻¹ for RK32 and RK119). Selection against the donor was done by omitting leucine, histidine, arginine and methionine from the medium. All the wild strains gave Sor⁺ transconjugants, at frequencies between 1 per 5×10^2 and 1 per 3×10^7 donors; for AB1621 the frequency was 1 per 3×10^2 . The conjugations were repeated using JC1553 strains carrying four different F primes: two carried *sorT*⁺*A* primes and two carried *sorTA*⁺ primes. Plates were incubated for 10 d at 30 °C. No Sor⁺ colonies developed.

The *sorT*⁺*A*⁺ prime was transferred to two strains of *Salmonella typhimurium*, one of *Shigella flexneri*, one of *Klebsiella aerogenes* and to two other unidentified wild strains of *Klebsiella*. The frequencies of transfer were from 1 per 5×10^5 to 1 per 10^8 donors. Sor⁺ colonies grew to about 2.5 mm diameter after 3 d at 37 °C, except that the *Shigella* colonies grew much more slowly to about 1 mm diameter.

DISCUSSION

The eight *sor* regions all occupied approximately the same positions in their respective donors, relative to genes within cotransduction distance, though the experiments would not reveal minor differences of gene order. Transduction of *sor* into *E. coli* K12 does not imply homology between the incoming *sor* genes and the corresponding region in K12, but indicates some homology in regions extending about 2 min on either side of *sor*. For different wild donors the frequencies of transduction were rather similar, as were the frequencies of cotransduction and the frequencies in exchanges between K12 and K12 (*sor*⁺) hybrids, indicating that the homology between wild donors and K12 was considerable. The *sor* genes themselves did not noticeably obstruct crossing-over with the K12 chromosome, suggesting that they were not associated with long tracts of DNA non-homologous with K12. If *sor* was associated with considerable non-homology, fragments transduced into K12 might persist as exogenote fragments. Of transductants selected to be Sor⁺ (Table 3), less than 10% gave evidence of instability for *sor*⁺, and they may have been partial diploids, or lysogens containing specialized transducing phage: the rest displayed recessive donor characters and must have contained haploid recombinant regions.

The requirement for gene *crp*⁺ shows that the *sor*⁺ genes are subject to catabolite repression control. This indicates compatibility of the *sor*⁺ control regions with K12 catabolite repression

protein, unless the successful transductions from wild strains entrain *sor*⁺ genes with K12 control regions.

The *Sor*⁺ character was generated by at least two closely linked genes; *sorT*⁺ probably specifies an L-sorbose permease and *sorA*⁺ an L-sorbose-1-phosphate reductase.

Kelker *et al.* (1972) showed that in *Klebsiella aerogenes*, L-sorbose catabolism requires a sorbitol-6-phosphate dehydrogenase activity. *Escherichia coli* K12 possesses an inducible sorbitol-6-phosphate dehydrogenase, encoded by the gene *srlD* (Lengeler & Lin, 1972). This may explain the fact that sorbitol annulled the partial inhibition of *Sor*⁻ bacteria by L-sorbose; sorbitol may have induced sorbitol dehydrogenase, thereby completing a pathway for L-sorbose metabolism. Lengeler (1977) reported that intracellular accumulation of fructose 6-phosphate is the cause of inhibition of *pfkA* mutants growing in the presence of fructose, sorbitol and mannitol. L-sorbose also inhibited *pfkA sor*⁺ strains, suggesting that it is also converted to fructose 6-phosphate.

Defects in catabolism of a carbohydrate, after phosphorylation and intracellular accumulation, often cause the carbohydrate to become inhibitory (Yarmolinsky *et al.*, 1959; Englesberg *et al.*, 1962; Solomon & Lin, 1972; Ferenci & Kornberg, 1973; Lengeler, 1977). Uridine and thymidine overcame the inhibition by L-sorbose, at concentrations indicative of action as substrates rather than cofactors, and must offer a clue about the mechanism of inhibition. Inhibition might result from incomplete or incorrect metabolism of L-sorbose by enzymes normally acting on other sugars, and might lead to irreversible combination of a nucleoside cofactor with L-sorbose, or a phosphorylated derivative, or to irreversible phosphorylation of a nucleotidyl transferase. Nucleosides might relieve the inhibition by replenishing the cofactor or serving as further acceptors of phosphate.

The *sac*⁺, *rtl*⁺, *atl*⁺ and *gat*⁺ genes, which are also found in some wild strains but not others, all have characteristic locations (Hill & Charles, 1980; Woodward & Charles, 1980*b*): the *sor*⁺ genes similarly occupy a characteristic region of the chromosome. One may conceive an ideal linkage map for a species, with each existing strain exhibiting only a sample of genes from the ideal map. To what extent the genes in wild strains may be transposed from their position in the ideal map is an important question for investigation.

If selective forces do not act to maintain linkage relations, then the expectation is that genes in different lineages will evolve different linkage relations. Genetic exchange may be the only force capable of conserving linkage relations in *E. coli*. The most likely mechanism of gene transfer, transduction and plasmid-mediated conjugation, could only conserve linkage relations over short distances. Genetic exchange need not be frequent, compared with asexual reproduction, provided that occasionally it conferred selective advantage on some progeny.

About 40% of the wild strains examined were *Sor*⁺. Similar polymorphisms exist for many characters. The frequencies observed in this laboratory are often surprisingly similar to those recorded by Edwards & Ewing (1972) for rather different circumstances. It seems increasingly likely that the proportions of positives and negatives may be balanced at particular ratios for given characters. Why do such polymorphisms exist? The positive and negative morphs of a given character might be selectively neutral, within sexually integrated populations. Alternatively, wild strains might constitute genetically isolated lineages with, for example, the *Sor* morphs being either neutral or adaptive. On the other hand, the genes generating the different morphs might flow through *E. coli* (and perhaps close relatives or even distinct organisms sharing the same ecological niches) as a result of sexual reproduction, providing balancing selective effects, either continuously or on different occasions. This would mean that the variation represents genetical polymorphism (Ford, 1971). If so, the possibility emerges of a fruitful synthesis between the study of populations of bacteria and that of populations of higher organisms.

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