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Resistance of *Escherichia coli* to Penicillins

IX. Genetics and Physiology of Class II Ampicillin-Resistant Mutants That Are Galactose Negative or Sensitive to Bacteriophage C21, or Both

KERSTIN G. ERIKSSON-GRENNBERG, KURT NORDSTRÖM, AND PER ENGLUND

Department of Microbiology, University of Umeå, S-901 87 Umeå 6, Sweden

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Ampicillin-resistant mutants of class II are determined by a doubling of chromosomally and episomally mediated ampicillin resistance on agar plates. Several mutants were isolated from a female as well as from an Hfr strain. The mutants differed from each other in various properties such as response to colicin E2 and sodium cholate, response to the phages T4 and C21, and fermentation of galactose. By conjugation and transduction experiments, it was shown that mutations in at least four loci gave the class II phenotype. The mutations were found to be in the *galU* gene, the *ctr* gene, and two new genes close to *mtl* denoted *lpsA* and *lpsB*. The carbohydrate compositions of the lipopolysaccharides of the mutants were investigated and found to be changed compared to the parent strains. *GalU* mutants lacked rhamnose and galactose and had 11% glucose compared to the parent strain. The *lpsA* mutant also lacked rhamnose and had only traces of galactose and 58% glucose, whereas the *lpsB* mutant contained 14% rhamnose, traces of galactose, and 81% glucose compared to the parent strain.

Several genes are involved in the determination of ampicillin resistance in *Escherichia coli*. The gene *ampA* gives a 10-fold increase in ampicillin resistance (4) and in penicillinase activity (16). This gene is located close to *purA* (5), and the corresponding mutants are of class I type. In class II mutants, penicillinase-mediated resistance is increased twofold without any increase in penicillinase activity (25). In this paper a number of class II mutants are described. They fall into several subclasses with respect to their phenotypic properties. A member of one subclass has been investigated (26). Some of the properties studied offer better criteria for selection than the twofold increase in ampicillin resistance. Genetic experiments show that mutations in at least four unlinked loci can give the class II phenotype. Some of these mutants were found to be galactose negative. The catabolism of galactose and some other sugars is shown in Fig. 1. The galactose and glucose moities of lipopolysaccharide are derived from two intermediates of the galactose catabolism, uridine diphosphate galactose (UDPgal) and uridine-5'-diphosphoglucose (UDPG) as is also indicated in Fig. 1.

Galactose-negative mutants can be obtained by mutation in a number of genes. Some of these mutants will also be deficient in UDPG or UDPgal, or both, i.e., *galE*, *galU*, *pgi*, and phosphoglucomutase-negative mutants. These can be distinguished since *galU* mutants will always be UDPG and UDPgal deficient whereas the other mutants will contain UDPG and UDPgal in the presence of galactose (*galE*), maltose or glucose (*pgi*), and maltose (phosphoglucomutase deficient).

Furthermore, mutants of some of these types are known to have lipopolysaccharide which is deficient in galactose and, in some cases, in glucose. Similar effects may be obtained by mutations in pathway 4 in Fig. 1. Bacteria deficient in galactose in their lipopolysaccharide have been reported to be host for phage C21 (34). In this paper, we present evidence that the class II phenotype can be obtained by mutations in *galU* and in two genes presumably belonging to pathway 4 (*lpsA* and *lpsB*).

MATERIALS AND METHODS

Organisms. The *E. coli* K-12 strains used and their characters are given in Table 1. The R factor, R1, mediating resistance to ampicillin, chloramphenicol, sulfonamides, streptomycin, and kanamycin, was obtained from N. Datta (19). *Salmonella typhimurium* SL869 and the phage C21 were given to us by A. Lindberg, Stockholm. The two phages T4 and T6 were propagated on strain G11. ϕW is the phage ϕII described by Wollman (20, 21). Phage T1, grown on *E. coli* B, was obtained from G. Bertani, Stockholm. Phage P1*bt* was grown as described by Eriksson-Grennberg (5). Phage AK6 was isolated from sewage as described below.

Media. Minimal medium was made from the basal

medium E of Vogel and Bonner (45) supplemented with 0.2% glucose, thiamine (1 μg/ml), the required amino acids (25 μg of the L-epimer per ml), and adenine and uracil (10 μg/ml). The rich medium was the LB medium described by Bertani (1), but containing 0.2% glucose and usually supplemented with medium E and thiamine. In some experiments, glucose was omitted or replaced by other carbon sources. LA plates contained LB medium, 2.5×10^{-3} M $CaCl_2$ and vitamins as described by Eriksson-Grennberg (5). All plates contained 1.5% agar. Streptomycin was used for counterselection in some crosses, the plates being supplemented with 100 μg of streptomycin sulfate per ml. Galactose fermentation was tested on purple base agar (Difco) with 1% galactose added. Glucose tetrazolium indicator plates (TTC plates) were made as described by Fraenkel and Levisohn (6).

Growth conditions. Growth conditions were as described by Nordström et al. (26).

Measurement of cell size distribution. A Coulter counter, model B, was used to measure cell size distribution (cf. 27).

Transduction and conjugation experiments. Transductions were performed as described by Eriksson-Grennberg (5) but without allowing time for phenotypic expression. Conjugation experiments were performed by the method of Eriksson-Grennberg (5) for the crosses in Table 4; for the remaining experiments, we used the following gradient method, as described by de Haan et al. (9). The cells were grown logarithmically in LB medium, and about 5×10^7 donor cells per ml were mixed with 5×10^8 recipient cells per ml. After 5 min at 37 C, the mating mixture was diluted 100 times with prewarmed LB medium, and incubation was continued without agitation for another 3 hr to allow the complete transfer of the chromosome and recombinant formation from the zygotes to occur before plating on selective medium.

Materials. D-Ampicillin was kindly given to us by Astra, Södertälje, Sweden, and chloramphenicol and streptomycin sulfate by Kabi AB, Stockholm, Sweden. UDPG, niacinadenine dinucleotide (NAD) and uridine-5'-diphosphoglucose dehydrogenase (UDPG dehydrogenase; EC 1.1.1.22) were obtained from Sigma Chemical Co., St. Louis, Mo., galactose-*l*-^{14}C was from the Radiochemical Centre, Amersham, England.

Determination of UDPG. Cells were grown in LB medium. Exponentially growing cells were harvested and extracted with 10 ml of 70% ethanol at 70 C for 5 min. The ethanol was removed with ether. The water phase was freeze-dried and dissolved in 100 μliters of water. The UDPG content of the cell extract was determined by the method of Strominger et al. (36).

Preparation of cell walls; lipopolysaccharide extraction and hydrolysis; analyses of carbohydrate composition of the lipopolysaccharide. The bacteria were grown in LB medium on a rotary shaker to a density of 200 Klett units. The culture volume was 1 to 2 liters. The centrifuged cells were suspended in NaCl containing 10 μg of deoxyribonuclease per ml and disintegrated in a Sorvall Omni-Mixer using 0.17 to 0.18-mm ϕ glass beads (Braun, Melsunger, Germany). After centrifugation at 4 C at 23,500 × *g* for 30 min, the cell walls were washed with cold water. Lipopolysaccharide was extracted by the phenol-water method (44). The hydrolysis and analysis of the carbohydrate part of the lipopolysaccharide were done as described by Holme et al. (11) with the following modifications (T. Holme, *personal communication*). The sulfuric acid was neutralized with $BaCO_3$, the precipitate was filtered off, and sodium borohydride was added. Excess of this compound was destroyed by acetic acid. After removing the methyl borate with methanol, ethanol-toluene (1:1, v/v) was added instead of benzene. The samples were injected into a column of 5% ECNSS-M (Applied Science Laboratories, State College, Pa.) on Chromosorb W (80 to 100 mesh; Perkin-Elmer, Beaconsfield, England) in a Perkin-Elmer model 900 gas chromatograph. The column temperature was 200 C.

Uptake of galactose-1-^{14}C. Cells were grown in LB medium without glucose or, in the case of D21e18, in minimal medium with sodium pyruvate, to about 2×10^8 cells/ml. The cells were centrifuged and suspended in minimal medium without a carbon source. Galactose-*l*-^{14}C (5 μliters of a solution containing 50 μCi/ml; specific activity 55.7 mCi/mmole) was added to 2.5 ml of the bacterial suspension. At intervals during the first 6 min, samples (500 μliters) were filtered through Whatman GF/A filters and washed two times with 10 ml of cold minimal medium lacking a

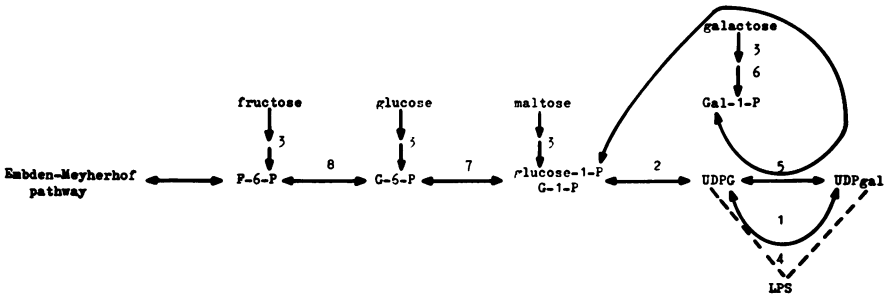

FIG. 1. *Biosynthetic pathway for UDPG and UDPgal. Reaction steps: 1, UDPgal 4-epimerase (EC 5.1.3.2), galE; 2, UDPG pyrophosphorylase (EC 2.7.7.9), galU; 3, carbohydrate transport, ctr; 4, function not known, lpsA, lpsB; 5, gal-1-P uridyltransferase (EC 2.7.7.12), galT; 6, galactokinase (EC 2.7.1.6), galK; 7, phosphoglucomutase (EC 2.7.5.1), gene not known; 8, phosphoglucoisomerase (EC 5.3.1.9), pgi. Abbreviations: F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; G-1-P, glucose-1-phosphate; UDPG, uridinediphosphoglucose; UDPgal, uridinediphosphogalactose; gal-1-P, galactose-1-phosphate; LPS, lipopolysaccharide.*

TABLE 1. *Escherichia coli K-12 strains used and their relevant characters*

Strain	Origin	Sex[a]	Ampicillin resistance		Response to *str*[d]	Response to phage T6[d]	Prophage λ[d]	Other important markers[d]
			Resistance class[b]	Phenotype[c]				
G11	Stent and Brenner (35)	HfrC	Wild type	amp-s	s	s	+	*ilv*, *metB*
G11a1	Eriksson-Grennberg et al. (4)	HfrC	Class I	amp-10	s	s	+	*ilv*, *metB*
G11e1	Eriksson-Grennberg et al. (4)	HfrC	Class II	amp-20	s	s	+	*ilv*, *metB*
G11e2-e6	This paper	HfrC	Class II	amp-20	s	s	+	*ilv*, *metB*
D21	Boman et al. (2)	F⁻	Class I	amp-10	r	r	+	*proA*, *trp*, *his*
D21e7-e13	This paper	F⁻	Class II	amp-20	r	r	+	*proA*, *trp*, *his*
D21e16-e19	This paper	F⁻	Class II	amp-20	r	r	+	*proA*, *trp*, *his*
HfrH	Hayes (10)	Hfr	Wild type	amp-s	s	s	−	
Hfr6		Hfr	Wild type	amp-s	s	s		
KL16	Low (17)	Hfr	Wild type	amp-s	s	s	−	*met*
AB311	Taylor and Adelberg (39)	Hfr	Wild type	amp-s	r	s	−	*thr*, *leu*
KL25	Low (17)	Hfr	Wild type	amp-s	s	s	−	
J4		Hfr	Wild type	amp-s	s	s		*thr*, *leu*
Jc12		Hfr	Wild type	amp-s	s			*purC*, *met*[e] *mtl*, *xyl*
X195	Pearce and Meynell (29)	F⁻	Wild type	amp-s	r			*pyrF*, *purB*, *trp*, *tyr*, *pro*, *his*, *met*
MS31	Burman and Nordström (3)	F⁻	Wild type	amp-s	r		+	*pyrD*, *trp*, *gal*
MS32	This paper[f]	F⁻	Wild type	amp-s	r		+	*pyrD*, *trp*

[a] Injection orders for the Hfr strains: HfrC, O-*purE-proB-thr*; HfrH, O-*thr-proB-trp*; Hfr6, O-*purE-trp-his*; KL16, O-*lysA-his-trp*; AB311, O-*his-trp-proB*; KL25, O-*ilv-ampA-thr*; J4, O-*pgi-metB-str*; Jc12, O-*argG-str-ampA*. The origins and injection orders are shown in Fig. 2.

[b] All class I and II strains contain the same allele of *ampA* (*ampA1*).

[c] For discussion of phenotype, see reference 25.

[d] Abbreviations: *amp*, ampicillin; *gal*, galactose; *his*, histidine; *ilv*, isoleucine-valine; *leu*, leucine; *lys*, lysine; *met*, methionine; *mtl*, mannitol; *pgi*, phosphoglucoisomerase; *pro*, proline; *pur*, purine; *pyr*, pyrimidine; *str*, streptomycin; *thr*, threonine; *trp*, tryptophan; *tyr*, tyrosine; *xyl*, xylose; r, resistance; s, sensitivity. The capital letters after some of the symbols refer to the genetic map of Taylor and Trotter (40).

[e] This strain is mutated in either *metA* or *metB*, as growth was obtained on cystathionine and homocysteine but not on homoserine.

[f] MS32 is a spontaneous galactose-positive revertant obtained from MS31.

carbon source. The filters were dried and counted in a Nuclear-Chicago liquid scintillation counter.

RESULTS

Isolation and characterization of class II mutants. The class II phenotype is defined by a doubling of episomally and chromosomally mediated ampicillin resistance on plates (25), and mutants were selected on LA plates containing ampicillin (4). G11e-strains were isolated from G11a1; D21e-strains were isolated from D21 (Table 1). The G11e strains G11e1–G11e5 and D21e7 were isolated on plates containing 50 μg of DL-ampicillin (ratio between the epimers 3:2) per ml, strains D21e8–12 on plates containing 50 μg of D-ampicillin per ml, strain D21e13 on a

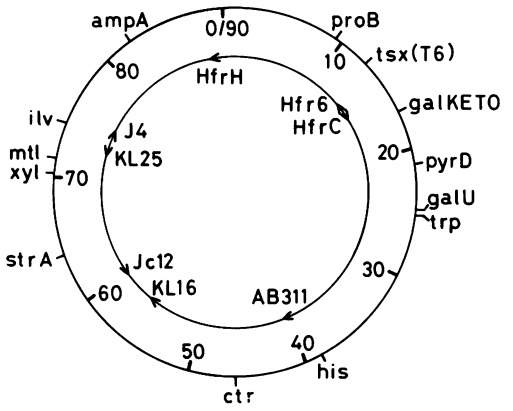

FIG. 2. *Map of the Escherichia coli K-12 chromosome according to Taylor and Trotter (40) with some markers and the origins and injection orders of the Hfr strains used.*

plate containing 30 μg of D-ampicillin per ml, and strains G11e6, D21e16, D21e17, D21e18, and D21e19 were isolated on plates containing 40 μg of D-ampicillin per ml. All mutants were spontaneous, and the frequency with which class II mutants occur is about 10^{-6} to 10^{-5}. The R factor, R1, was introduced into the mutants as previously described (25), and the ampicillin resistance of these R-factor strains was at least twice that of D21-R1 and G11a1-R1 (Table 2).

In a previous paper (26), the physiological properties of one class II strain (G11e1) were studied in detail. The mutation gave very pleiotropic effects on the phenotype; it affected the osmotic stability and the resistance to many drugs, conferred resistance to phages T4 and T3-1, sensitivity to cholate and ethylenediaminetetraacetate, and made the cells release a portion of their penicillinases. Burman and Nordström (3) found that the mutation also mediates tolerance to colicins E2 and E3. Some of the characters listed above were only slightly changed (by a factor of 2), and others were more drastically affected. We selected some of the latter for a close characterization of the mutants isolated. The results are summarized in Table 2, which shows that the mutants can be divided into a rather large number of subclasses, each with its specific set of changed properties. Some of the mutants were also found to have lost the ability to use galactose as the sole carbon source.

Phage C21 grows only on strains of *Salmonella* and *E. coli* that have a reduced amount of galactose in the lipopolysaccharide of the cell envelope (34). When growth of phage C21 was tested on the strains listed in Table 2, some of the mutants were found to be sensitive. We selected for further studies those class II mutants that are galactose negative, sensitive to C21, or both.

Sewage from a community was centrifuged, and samples (0.1 ml) were mixed with about 10^8 bacteria in soft agar. After incubation overnight, the plaques were counted. Strains D21e7, D21e8, and D21e10 were poor hosts for phages, whereas the other D21e strains gave about the same phage titer. Similarly, among the G11 strains, G11e5 gave low plaque counts. Stocks of phages were grown on the strains on which the phages were isolated initially. These stocks were spotted onto lawns of 10^8 bacteria in soft agar. Lysis was recorded after incubation overnight. Also in this test, D21e7, D21e8, and D21e10 were found to be poor hosts for phages. However, a few phage clones were able to differentiate between D21e7 on the one hand and D21e8 and D21e10 on the other. One phage clone, AK6, grew only on D21e7. This phage was used for the characterization of the bacterial mutants (Table 2).

Monner et al. (21) reported that the phage φW can distinguish between strains that are different in the cell envelope. We therefore used this phage to test the various class II mutants. As is shown in Table 2, phage φW grew only on strains D21e7 and D21e19, belonging to subclasses IIb-c.

To estimate the frequency of the different class II mutants, a number of additional spontaneous class II mutants were isolated and tested for response to cholate and phage C21 and for utilization of galactose (Table 3). A minority of the clones were galactose negative. These were all sensitive to cholate and phage C21 (in the presence of glucose, galactose, and maltose), and thus they resemble D21e8 in phenotype. A larger fraction of the clones were galactose positive and sensitive to phage C21; thus they resemble subclass IIb-c. One group of mutants was resistant to C21, galactose positive, and cholate sensitive (see subclass IIg in Table 2). The vast majority were galactose positive and resistant to cholate (see subclass IIe in Table 2). One group of mutants obtained was sensitive to phage C21 and resistant to cholate. This class has not yet been tested further.

The results of experiments with strains from some of the subclasses characterized in Table 2 follow.

Subclass IIa. The members of subclass IIa are galactose negative, sodium cholate sensitive, resistant to phage T4, and sensitive to phage C21 (see Table 2). They are also sensitive to phage P1bt (see Table 5).

TABLE 2. *Phenotypic properties of class II ampicillin-resistant mutants isolated from D21 and G11a1*

Subclass no.	Representative strains	D-Ampicillin resistance (μg/ml)[a]		Response to		Fermentation of galactose[c]
		-R1	-R1	Colicin E2[b]	Sodium cholate[a] (mg/ml)	
I (parental)	G11a1, D21	20	75	s	40	+
IIa	D21e8, D21e10, D21e16, D21e17	50	200	s	≤10	-
IIb	D21e7	50	250	s	≤10	+
IIc	D21e19	30-40	200	s	20	+
IId	D21e18	30-40	150	s	20	-
IIe	D21e9, D21e11, D21e12, D21e13	30-40	100-150	s	40	+
IIf	G11e5, G11e6	40	250	tol	≤10	-
IIg	G11e1-e4	40	200	tol	≤10	+

Subclass no.	T4, time to lysis (min)[d]	EOP for phage C21 on agar plates with			EOP for phages		Mutated genes
		Glucose	Galactose	Maltose	AK6	φW	
I (parental)	25	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-6}$	$<10^{-7}$	
IIa	∞[e]	1	1	1	$<10^{-6}$	$<10^{-7}$	*galU*
IIb	25	1	1	1	1	1	*lpsA*
IIc	25	1	1	1	1	1	*lpsB*
IId	25	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-6}$	$<10^{-7}$	*ctr*
IIe	25	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-6}$	$<10^{-7}$	?
IIf	25	1	$<10^{-8}$	1	$<10^{-6}$	$<10^{-7}$	?
IIg	60	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-6}$	$<10^{-7}$	*tolD* (in G11e1)

[a] Single-cell tests on LA plates. Resistance is given as the highest concentration in the plates at which all cells plated gave rise to colonies. About 200 cells were spread and the plates were incubated overnight (25).

[b] Log cells (4×10^8) in LB medium were mixed with about 2×10^9 killing units of colicin E2 [prepared as described by Nagel de Zwaig and Luria (22)]. After 10 min at 37 C, viable count was determined. Survival of the sensitive (s) clones was about 1%. The G11e strains survived to about 50% and these were tested for adsorption by centrifuging the cell-colicin suspension and assaying the supernatant fluid for colicins with G11a1 used as test organism. If there is no colicin left in the medium, the strain is tolerant (tol; reference 24).

[c] Fermentation of galactose was tested on purple base agar containing galactose.

[d] Logarithmically growing cells were diluted in LB medium. Phages were added at a multiplicity of 5, and optical density was measured. The period between the addition of phages and the highest value on the growth curve is defined as time to lysis.

[e] Resistance to T4 was also tested on plates by using a high concentration of phages (10^8 per plate). No plaques were obtained.

Since preliminary conjugation experiments showed that the class II mutation in D21e8 was located in the *pro-trp* region, the crosses shown in Table 4 were performed. All these conjugation experiments indicated that the mutation was closely linked to *trp*. The transduction experiments shown in Table 5 were then done. D21e8 was used as recipient for P1(G11a1), and 56% of the Trp^+ transductants had acquired the class I phenotype (experiment 2). As in the case of the conjugations, we found that all class II properties tested for were lost in one single event. Of seven D21e strains tested, two showed cotransduction between *trp* and the class II mutation (experiments 1 to 7). In transductions 8 to 11, some D21e strains were used as donors and X195 as recipient. $PyrF^+$ transductants were selected, and only strains D21e8 and D21e10 showed cotransduction between *pyrF* and the class II muta-

TABLE 3. *Phenotypic properties of spontaneous class II mutants*[a]

No. of mutants isolated from strains		Phenotypic properties		
D21	G11a1	Fermentation of galactose	Response to C21	Response to cholate
7	7	-	s	s
29	11	+	s	s
5	1	+	s	r
7	0	+	r	s
40	69	+	r	r

[a] The mutants were isolated on LA plates containing 40 μg of D-ampicillin per ml. They were tested for the phenotypic properties by replica plating on galactose-purple base agar, on LA plates seeded with phage C21, and on LA plates containing 40 mg of sodium cholate per ml.

TABLE 4. *Analyses of crosses with strain D21e8*

Expt no.	Cross	No. of recombinants tested	Selection	Class I phenotype (%)[a]	Trp+ (%)
1	HfrH × D21e8	65	Cholate^r/str^r	100	58
		47	Pro+/str^r	2	2
		50	Trp+/str^r	90	
2	Hfr6 × D21e8	63	Cholate^r/str^r	100	48
		71	Trp+/str^r	82	
3	AB311 × D21e8	96	Trp+/Thr+Leu+	90	

[a] The recombinants were tested for several properties characteristic for the class II phenotype. There was no segregation of these properties in any of the conjugation experiments.

TABLE 5. *Transduction experiments using phage P1bt*

Expt no.	Donor	Recipient	Selection	No. of transductants tested	Relative frequency (% of selected property)			
					Trp+	PyrF+	Ampicillin resistance class[a]	
							I	II
1	G11a1	D21e7	Trp+	99			0	
2	G11a1	D21e8	Trp+	246			56	
3	G11a1	D21e9	Trp+	99			0	
4	G11a1	D21e10	Trp+	99			45	
5	G11a1	D21e11	Trp+	97			0	
6	G11a1	D21e12	Trp+	95			0	
7	G11a1	D21e13	Trp+	99			0	
8	D21e8	X195	PyrF+	172	74			7
9	D21e9	X195	PyrF+	224				0
10	D21e10	X195	PyrF+	95				9
11	D21e13	X195	PyrF+	215				0
12	G11e1	X195	Trp+	60		41		0
			PyrF+	62	58			0
			PurB+	31	0			0
13	G11e1	D21	Trp+	56				0
14	G11e2	D21	Trp+	87				1
15	G11e3	D21	Trp+	86				0
16	G11e4	D21	Trp+	87				0
17	G11e5	D21	Trp+	36				0
18	G11e1	D21e8	Trp+	214			49	

[a] Ampicillin resistance and several other phenotypic properties characteristic for the class II phenotypes were tested. There was no segregation of these properties in any of the transductions.

tion. Strains D21e7, D21e9, D21e11, D21e12, and D21e13 did not show any cotransduction between *trp* or *pyrF* and the class II mutation; neither did the five G11e strains tested (experiments 12 to 17). One of the G11e strains was used as donor for transduction with D21e8 as recipient, and the cotransduction frequency between *trp* and the class II mutation was found to be 49% (experiment 18).

The transduction data show that the *trp* gene is located between the class II mutation in D21e8 and D21e10 and the *pyrF* gene. The gene *tonB* mediating sensitivity or resistance to phage T1 and some colicins is located in the same region as the class II mutation. All class II mutants were therefore tested for resistance to phage T1. All were found to be sensitive.

Some of the phenotypic properties described in Table 2 could be expected to be well suited for studies of reversions. Selection for cholate-resistant as well as galactose-positive revertants from D21e8 and D21e10 was done. No revertants were found when 2×10^{10} cells were plated.

Thus strains D21e8 and D21e10 seem to contain a deletion covering the *galU* gene. Therefore we tried to isolate class II mutants with a point mutation in the *galU* gene. Strain D21 was

spread on LA plates containing 40 μg of D-ampicillin per ml. Resistant colonies appeared with a frequency of about 5×10^{-6}, measured per input number of cells. Of 88 clones, 9 proved to be galactose negative and sensitive to phage C21 even in the presence of galactose, i.e., they showed the same phenotype as *galU* strains. They were also found to be resistant to phage T4 and sensitive to sodium cholate. The clones were tested for Gal$^+$ reversion, and seven of the clones were leaky, since they gave a thin lawn of bacterial growth on galactose plates. However, two clones, D21e16 and D21e17, gave no background growth, but galactose-positive colonies were obtained with a frequency of about 10^{-7} to 10^{-6}. The galactose-positive revertants of D21e16 and D21e17 were of class I phenotype in every respect tested.

The map position of the class II mutation in strains D21e16 and D21e17 was tested by crossing these females with the Hfr strain KL16. Of the recombinants selected as Trp$^+$, most (95% from D21e16 and 90% from D21e17) had the class I phenotype. Thus, the class II mutation in strains D21e16 and D21e17 is linked to *trp*.

Strains D21e8 and D21e10 are galactose-negative mutants. Incorporation experiments with galactose-*1*-^{14}C showed that D21e8 and D21e10 were not defective in the uptake of galactose.

Because it has been reported that some galactose-negative mutants are sensitive to galactose (23, 37), we performed the experiment shown in Fig. 3. Strains D21 and D21e8 were grown in LB medium (without glucose) with and without galactose. D21e8 showed a marked reduction in growth rate in the presence of galactose. However, the cultures did not lyse. Viable counts were constant for several hours for D21e8 in the late-logarithmic growth phase. The size distribution of the cells was measured in a Coulter counter, and no tendency to filament formation was observed. This indicates that there was some killing and perhaps also lysis of individual cells in the presence of galactose. The same results were obtained with D21e10.

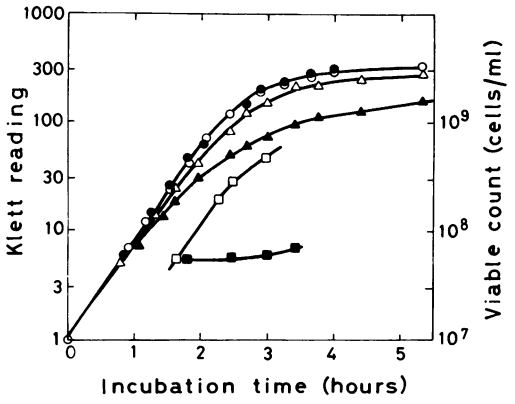

FIG. 3. *Effect of galactose on growth in LB medium (without glucose). The cells were pregrown in LB medium without glucose to a cell density corresponding to 100 Klett units. At zero time, the cells were diluted 100 times into prewarmed LB medium with (closed symbols) and without (open symbols) galactose. Optical density for D21 (circles) and for D21e8 (triangles) and viable count for D21e8 (squares) were measured at intervals.*

On LA plates containing galactose instead of glucose, D21e8 gave slow-growing colonies. When about 10^8 cells were plated on these plates, about 100 large colonies were obtained on a lawn of confluent growth. These galactose-resistant clones retained all class II properties (48 clones tested).

Since we suspected, from the C21 tests shown in Table 2, that strains D21e8 and D21e10 were *galU* mutants, we determined the UDPG content of the cells. The parent strain D21 contained 5 to 6 mμmoles of UDPG per 10^{10} cells, whereas the mutants D21e8 and D21e10 contained less than 0.5 nmole of UDPG.

Subclasses IIb and IIc. Members of the subclasses IIb and IIc are sensitive to phage C21 in spite of their ability to use galactose as the sole carbon source. However, the two subclasses differ in response to sodium cholate (Table 2).

To map the class II mutations in strains D21e7 and D21e19, a set of crosses was performed by using Hfr strains with different origins and injection directions. All the His$^+$, Trp$^+$, and Pro$^+$ recombinants selected in crosses with HfrH, HfrKL16, and HfrKL25 as donors retained the class II phenotype. Streptomycin was used for counterselection in these crosses. However, when Hfr strain J4 was crossed with D21e7 and His$^+$ recombinants were selected by using T6 resistance and Thr$^+$Leu$^+$ for counterselection, some of the recombinants were found to be of the class I phenotype. Out of 169 recombinants tested, 147 were streptomycin resistant, and 10 of these had lost the class II phenotype. Of the 22 recombinants which were streptomycin sensitive, 13 had lost the class II phenotype. To more closely relate the mutated gene (*lpsA*) in D21e7 to already known genes, a conjugation experiment was done with the Hfr strain Jc12 and D21e7. Pro$^+$ recombinants were selected with PurC$^+$ for counterselection. The 264 recombinants picked were tested for the following unselected markers: *str*, *xyl*, *mtl*, and *lpsA*, the mutated gene in D21e7. The results of this conjugation are summarized in Table 6. The frequency of crossing-over between the unselected genes

TABLE 6. *Analysis of crossing-over between unselected markers for Pro⁺ recombinants in crosses between Hfr Jc12 and the F⁻ strains D21e7 and D21e19*

Crossing-over type	Unselected markers[a]				Jc12 × D21e7		Jc12 × D21e19	
	str	*xyl*	*mtl*	*lpsA* or *lpsB*[b]	No. of recombinants	Frequency (%)	No. of recombinants	Frequency (%)
Hfr	s	−	−	+	50	0.18	42	0.18
F⁻	r	+	+	−	86	0.33	64	0.28
1	s	+	+	−	20	0.21	12	0.13
	r	−	−	+	35		18	
2	s	−	+	−	1	0.03	3	0.04
	r	+	−	+	8		7	
3	s	−	−	−	2	0.17	3	0.27
	r	+	+	+	43		60	
1+2	s	+	−	+	2	0.01	2	0.01
	r	−	+	−	1		1	
1+3	s	+	+	+	6	0.03	9	0.04
	r	−	−	−	3		1	
2+3	s	−	+	+	3	0.01	2	0.01
	r	+	−	−	0		0	
1+2+3	s	+	−	−	1	0.02	2	0.02
	r	−	+	+	3		3	

[a] In the conjugations, Pro⁺/PurC⁺ recombinants were tested for the unselected markers *str*, *xyl*, *mtl*, and *lpsA*/*lpsB*. Crossing-over type 1 represents crossovers between *str* and *xyl*, type 2 between *xyl* and *mtl*, and type 3 between *mtl* and *lpsA*/*lpsB*. Gene orders other than *str-xyl-mtl-lpsA*/*lpsB* between the markers were tried but did not fit the data.

[b] The marker *lpsA* was identified as cholate sensitivity, ampicillin resistance, and sensitivity to phages C21 and φW and the marker *lpsB* as ampicillin resistance and sensitivity to phage C21. In no case was there any segregation between these properties.

best fitted the gene order *str-xyl-mtl-lpsA*. Also, in the conjugation experiment with Jc12 and the F⁻ strain D21e19, a similar result was obtained (Table 6), and the data indicated that the gene order is *str-xyl-mtl-lpsB*, the latter being the mutated gene in D21e19.

In transduction experiments with strains D21e7 and D21e19 as donors and G11a1 as recipient, Ilv⁺ transductants were selected. None out of 200 transductants with D21e7 used as donor and none out of 200 transductants with D21e19 used as donor had received the *lpsA* or *lpsB* gene, respectively. Strain Jc12 was used as recipient and D21e7 as donor in a transduction. When 236 Xyl⁺ transductants were scored, 12.5% cotransduction between *xyl*⁺ and *mtl*⁺ 4.5% cotransduction between *xyl*⁺ and *lpsA* were found. Out of the *xyl*⁺-*lpsA* cotransductants, 2.5% also had the *mtl*⁺ gene. In a corresponding transduction with D21e19 used as donor, 343 transductants were investigated. The cotransduction between *xyl*⁺ and *mtl*⁺ was 11% and that between *xyl*⁺ and *lpsB* was 1%. All the transductants which had the *lpsB* gene also were *mtl*⁺.

Subclass IId. Tests of the phenotypic properties of D21e18 showed that this strain was galactose negative and resistant to phage C21 (Tables 2 and 7). This mutant was also unable to grow in minimal medium with glucose as sole carbon source. These properties indicated a defect in the carbohydrate transport system. Incorporation of galactose-*1*-^{14}C into cells of D21e18 and D21 grown in sodium pyruvate-minimal medium showed that the uptake was 1,450 counts/min for D21; the corresponding figure for D21e18 was 100 counts/min after 3 min (Fig. 4). In a conjugation experiment between HfrKL16 and D21e18, glucose⁺ recombinants were selected with streptomycin for counterselection. Among 88 recombinants, 22 had received the *his*⁺ gene. In an interrupted conjugation experiment with the same Hfr strain, His⁺ recombinants (sodium pyruvate was used as carbon source in the plates) and glucose⁺ recombinants were selected with streptomycin for counterselection. This cross showed that the wild-type allele of the mutated gene in D21e18 was injected 5 to 10 min before the *his*⁺ gene.

Subclass IIe. The members of subclass IIe had the same double resistance to ampicillin as all class II mutants, but they were wild type in all other properties shown in Table 2. Some transduction experiments were done (Table 5) which showed that the mutated gene in these strains is not located in the same region of the chromosome as *galU*. Members of this subclass were not studied further.

Subclass IIf. Strains G11e5 and G11e6 of sub-

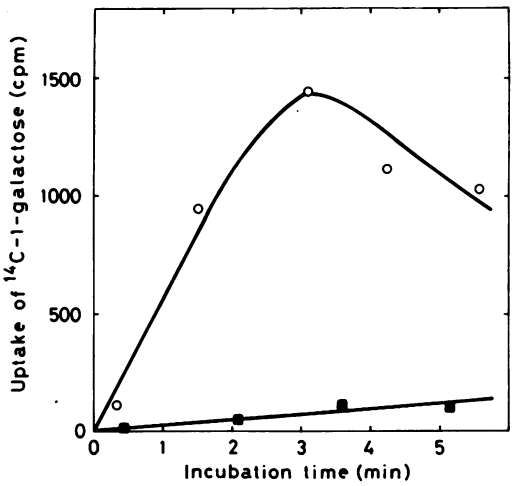

FIG. 4. *Uptake of galactose-1-^{14}C by strains D21 (○) and D21e18 (■). The cells were grown in minimal medium with sodium pyruvate as carbon source. After centrifugation and resuspension in minimal medium, galactose-1-^{14}C was added. At intervals, samples were taken, filtered, and washed. The dried filters were counted, and the values obtained were corrected for background.*

class IIf are sensitive to sodium cholate and resistant to phage C21 on galactose plates but are sensitive on other plates (Table 2). When fermentation of galactose was tested on purple base agar, the single-cell colonies did not change the indicator (Table 2). These strains are discussed elsewhere (*manuscript in preparation*).

Subclass IIg. Strain G11e1 of subclass IIg has been discussed by Nordström et al. (26) and Burman and Nordström (3). No further results are reported here.

Phosphoglucomutase-negative and phosphoglucoisomerase-negative mutants. As can be seen from the scheme shown in Fig. 1, there are strains other than *galU*- and *galE*-defective strains that should form a reduced amount of UDPgal, produce an altered lipopolysaccharide, and thus be sensitive to phage C21. Mutants defective in phosphoglucomutase should be rescued from killing by C21 in the presence of maltose (33). Mutants lacking phosphoglucoisomerase (*pgi*) would probably be sensitive to phage C21 in the absence of glucose but resistant in the presence of glucose and maltose. We tried to isolate both of these types of mutants by the following methods.

We attempted to isolate phosphoglucomutase-negative mutants by growing D21 in LB (without glucose) containing 0.2% maltose. At a cell density of 2×10^8 cells per ml, phage C21 was added at a multiplicity of about five to kill all C21-sensitive cells. The cells were then spead on LA plates containing 40 μg of D-ampicillin per ml. All clones tested (200) were found to be galactose positive and no mutase-negative mutants were found.

To select phosphoglucoisomerase-negative mutants, strain D21 was grown in LB medium without glucose and spread on LA plates with and without glucose and containing 40 μg of D-ampicillin per ml. A number of clones were tested on TTC plates and also for galactose utilization and sensitivity to phage C21. The results are summarized in Table 7. One clone (D21e18) was found to form red colonies on TTC plates. This strain was described above.

Fraenkel and Levisohn (6) have described the isolation of isomerase-negative mutants by spreading cells on TTC plates after mutagenic treatment and picking the red mutant colonies. We spread D21 cells (without mutagenic treatment), grown in LB medium, to a cell density of 4×10^8 cells per ml on TTC plates. However, we did not find any red colonies.

Carbohydrate content of the lipopolysaccharide. Strains which are mutated in the *galE* and *galU* genes have a reduced amount of galactose in their lipopolysaccharide (7, 13), whereas *galU* mutants also have reduced contents of glucose and rhamnose (13). Lipopolysaccharide from strains MS31, MS32, D21, D21e8, D21e7, D21e19, and D21e18 was prepared, and the sugar content was analyzed by gas chromatography. Table 8 shows the results. The *galE* mutant MS31 showed about 50% reduction of galactose compared to the wild-type strain MS32. The *galU* mutant D21e8 lacked both rhamnose and galactose and had a drastic reduction of the glucose content. The *lpsA* mutant D21e7 also had lost all its rhamnose and contained very little

TABLE 7. *Properties of spontaneous mutants isolated on LA plates containing 40 μg of D-ampicillin per ml*

Properties	No. of clones		
	G11a1	D21 pregrown in LB medium	
		+ Glucose	− Glucose
Total no. tested	350	500	300
Red on TTC plates ..	0	0	1[a]
Galactose negative[b] ..	26	16	0
C21 sensitive	54[c]	101	14

[a] This strain (D21e18) was further tested and found to be defective in the uptake of carbohydrates (*ctr* mutant).

[b] Fermentation of galactose was tested on purple base agar containing galactose.

[c] One clone (G11e6) was galactose negative and sensitive to phage C21 in the absence of galactose but resistant to C21 in the presence of galactose.

galactose, whereas the *lpsB* mutant D21e19 retained some rhamnose and contained very little galactose. Strain D21e18 of subclass IId had lipopolysaccharide of normal composition.

DISCUSSION

Identity and map positions of the class II mutations. The conjugation and transduction experiments with strains D21e8 and D21e10 of subclass IIa show that the mutation in these strains was linked to the *trp* gene and to a lesser extent to *pyrF*. The cotransduction data show that the *trp* gene lies between *pyrF* and the class II mutation. In this part of the chromosome, the *tonB* gene is known to give pleiotropic surface effects. Since D21e8 and D21e10 are sensitive to phage T1, the class II mutation is not likely to be allelic to the *tonB* gene. Furthermore, D21e8 and D21e10 are galactose negative.

The *galU* gene is also linked to *trp* (33). The physiological and enzymological data for D21e8 and D21e10 show that sensitivity to galactose and phage C21 (Table 2), the amount of UDPG in the cells, and the glucose and galactose content of the lipopolysaccharide (Table 8) are affected in the same way as in *galU* mutants. These studies of the class II mutation show that strains D21e8 and D21e10 are mutated in the *galU* gene.

However, the reversion studies favor the hypothesis that the class II mutation in strains D21e8 and D21e10 is a deletion. Deletions are common in the *trp-tonB* part of the chromosome (8). Therefore, strains D21e16 and D21e17 were isolated. These strains are presumably also *galU* mutants, as they all have the same class II phenotype as D21e8 and D21e10, and the mutations are located close to *trp*. However, galactose-positive revertants from D21e16 and D21e17 could be obtained, which shows that these strains contain point mutations in the *galU* gene. Since these revertants had lost the class II phenotype, it is likely also that in D21e8 and D21e10 the class II phenotype is due to deletions in the *galU* gene and not to deletion of other gene(s) closely linked to the *galU* gene.

Strains D21e7 and D21e19 of subclasses IIb and IIc were sensitive to phage C21, although these strains can use galactose as carbon source. Conjugation and transduction experiments (Table 6) show that the class II mutations in D21e7 and D21e19 are not located in the same part of the chromosome as the mutations in the C21-sensitive and galactose-negative mutants. The linkage data obtained in the conjugations with Hfr strain Jc12 showed that the *lpsA* gene in D21e7 and the *lpsB* gene in D21e19 are located in the *mtl* region (Table 6). The genes are denoted *lpsA* and *lpsB* because the properties of the lipopolysaccharide were changed (Table 8). The order of the genes is presumably *str-xyl-mtl-lpsA(lpsB)*. Using Hfr strains with different origins and orders of injection, we concluded that the class II mutations (*lpsA* and *lpsB*) in D21e7 and D21e19 are located between 55 and 74 min on the K-12 chromosome. Altogether, these results restrict the region in which the genes *lpsA* and *lpsB* can be located from 71 min (*mtl*) to 74 min (origin for HfrKL25).

TABLE 8. *Carbohydrate composition of lipopolysaccharide (LPS) from different mutants*

Strain	Ampicillin subclass	Carbohydrate (μg of LPS/mg)			
		Rhamnose	Galactose	Glucose	Heptose
MS32	Wild type	9.6	28.5	73.0	94.3
MS31	Wild type (*galE*)	9.1	12.5	52.5	72.2
D21	I	14.3	28.5	77.6	94.8
D21e8	IIa (*galU*)	0.0	0.0	8.3	41.2
D21e7	IIb (*lpsA*)	0.0	1.0	45.8	47.0
D21e19	IIc (*lpsB*)	2.3	0.7	63.2	90.0
D21e18	IId (*ctr*)	15.1	34.6	103.0	122.5

The transduction data obtained showed 12.5 and 11% cotransduction between *xyl* and *mtl* when D21e7 and D21e19, respectively, were used as donors. The cotransduction between *xyl* and *lpsA* was 4.5% and between *xyl* and *lpsB* the corresponding figure was 1%, which confirms the gene order *xyl-mtl-(lpsA, lpsB)-ilv*.

It was found that the carbohydrate composition of the lipopolysaccharide in D21e7 and D21e19 was different from that of the parent strain (Table 8). Rhamnose and galactose were lacking and, in addition, the glucose content was reduced compared to that of strain D21. Strains D21e7 and D21e19 are galactose positive; therefore, we propose that the gene products of the *lpsA* and *lpsB* genes affect some step in the incorporation of galactose (from UDPgal) and glucose (from UDPG) into the complete lipopolysaccharide structure. We have not yet tried to determine which enzymes are defective in D21e7 or D21e19.

The genes *lpsA* and *lpsB* are located close to each other. There is some difference in the phenotype of D21e7 (*lpsA*) and D21e19 (*lpsB*) (see Table 2, ampicillin and cholate resistance). This difference is also reflected by a difference in the composition of the lipopolysaccharide of the two strains (Table 8). Therefore, it seems logical to denote the mutation in strains D21e7 and D21e19 by different gene symbols, *lpsA* and *lpsB*. Schmidt et al. (32) reported that rough mutants of *E. coli* 08:K27 which are unable to

form a complete core in their lipopolysaccharide are mutated in genes in the region *str-met*. By analogy to work on *Salmonella*, these authors used the gene symbol *rfa*. In *S. typhimurium*, a number of *rfa* genes are located rather close to *mtl*, and one *rfa* gene is located between *ilv* and *metB* (31), i.e., in the same region as *lpsA* and *lpsB* in *E. coli* K-12. We have avoided using the gene symbol *rfa* because we have not used morphological properties in describing the mutants. Recently Tamaki et al. (38) reported that an enduracidin- and bacitracin-supersensitive mutant is mutated in a gene close to *xyl*. However, this mutant did not show any defect in the lipopolysaccharide.

During the search for phosphoglucomutase-negative mutants, the utilization of glucose was tested on TTC plates. One red mutant (D21e18) of subclass IId was obtained (Table 7). Growth experiments and uptake experiments using galactose-*1*-^{14}C (Fig. 4) showed that the strains were affected in the transport of carbohydrates. The conjugation experiments showed that the mutated gene is located between the origin of KL16 (55 min) and the *his* gene (38.5 min). Strain D21e18 is probably similar to the *ctr* mutants previously described (41). It has been shown that some of these mutants have a nonfunctioning enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system; however, according to Wang et al. (42), this cannot explain all the pleiotropic properties of the *ctr* mutants. Since knowledge about the carbohydrate transport system is very incomplete, it is difficult to explain why strain D21e18 is a class II mutant. However, since the transport of carbohydrates into the cell must involve several surface functions, it is reasonable that changes in the composition of the lipopolysaccharide or other parts of the cell envelope can affect the uptake of substances.

Formation of UDPG and UDPgal and the incorporation of glucose and galactose into lipopolysaccharide. In Fig. 1 we summarized the formation of UDPG and UDPgal from noncarbohydrate sources and from various carbohydrates. We have also indicated the transfer of glucosyl and galactosyl units into lipopolysaccharide (reaction 4 in Fig. 1). The latter process involves several steps (12, 18, 28).

From Fig. 1 it can be suggested that mutations in several genes (reactions 1, 2, 4, 7, and 8) should affect the carbohydrate composition of the lipopolysaccharide when the cells are grown in the absence of any carbohydrate. However, we have not yet been able to find any phosphoglucomutase (reaction 7) or phosphoglucoisomerase (reaction 8) mutants. We have received a *pgi*-mutant of *E. coli* K-12 and its corresponding wild type from Fraenkel and tested it for class II properties. However, the results were negative, since *pgi*-mutants must be grown in minimal medium without carbohydrate to give a lipopolysaccharide that is deficient in glucose and galactose. It is not possible to detect easily the class II phenotype when the cells are grown in minimal media with poor carbon sources such as glycerol. To our knowledge, no phosphoglucomutaseless mutants have been isolated from *E. coli*.

In Table 9, we have summarized the results obtained with mutants defective in reactions 1, 2, and 4.

GalE, *galT*, and *galU* mutants are sensitive to galactose because they accumulate galactose-1-phosphate (37). These mutants can be made galactose resistant by mutations in the *galK* gene (37). Strains D21e8 and D21e10 were sensitive to galactose (Fig. 3), and galactose-resistant clones could be isolated. These were all of class II phenotype, i.e., they were mutated in a site other than the *galU* region.

Strains D21e7 and D21e19 were isolated as being ampicillin resistant, and these strains turned out to be defective in the transfer of carbohydrate into lipopolysaccharide. Since several genes must be involved in this process, ampicillin resistance seems to be a criterion for screening for mutants that are defective in the various functions indicated as reaction 4 in Fig. 1. Mutants of this group are rather common among the class II mutants (Table 3).

As can be seen from Table 9, the mutants with a reduced amount of galactose in their lipopolysaccharide are sensitive to phage C21. Galactose need not be completely lacking to give C21 sensitivity. The *galE* mutants are reported to have retained 50% of the normal amount of galactose; nevertheless, they are sensitive to phage C21 (13, 30).

Weidel et al. (43) described T4-resistant mutants. These mutants lack heptose in their lipopolysaccharide. Strains D21e8 and D21e10 are resistant to phage T4, but their lipopolysaccharide contains heptose.

However, T4 resistance in *galU* mutants is not due to lack of absorption (*unpublished data*) but is due to restriction of the phage DNA which is not glucosylated in *galU* strains since these lack UDPG (34). Thus, T4 resistance does not necessarily depend on the absence of heptose in the lipopolysaccharide. The lipopolysaccharide of mutants D21e8 and D21e-10 lacks galactose and rhamnose and has a reduced amount of glucose. However, it is not possible to draw any conclusions from these data about the nature of the receptors for phage T4.

A reduction of the galactose content of the lipopolysaccharide is not enough in itself to

TABLE 9. *Phenotypic properties of mutants with galactose-defective lipopolysaccharide (LPS)*

Strain	Mutated gene	LPS composition (% of LPS of parent strain)				Phenotype			
		Rhamnose	Galactose	Glucose	Heptose	Ampicillin resistance class	Response to cholate[a]	Fermentation of galactose	Response to phage C21
D21/MS32	Wild type	100	100	100	100	I/Wild type	r	+	r
MS31	*galE*	100	45	72	77	Wild type	r	−	s
D21e8	*galU*	0	0	11	52	IIa	s	−	s
D21e7	*lpsA*	0	3	58	52	IIb	s	+	s
D21e19	*lpsB*	14	3	81	95	IIc	i	+	s

[a] The resistant (r) strains grew on plates (single cell tests) with 40 mg of sodium cholate per ml, the intermediate (i) resistant strain grew on 20 mg/ml, and the upper limit for the sensitive (s) strains was 5 to 10 mg/ml.

cause class II ampicillin resistance and sensitivity to sodium cholate. Strain MS31, which is a *galE* mutant, has neither of these class II properties. Both these phenotypic properties can be explained by some other changes of the cell envelope.

Pleiotropic effects of the class II ampicillin-resistant mutations. All the ampicillin-resistant class II mutants were selected on LA plates containing ampicillin. Some of these mutants showed only an altered response to ampicillin (Table 2), whereas other mutants showed a pleiotropic phenotype. It can be concluded from the above discussion that the same phenotypic response to ampicillin can be due to different genotypic changes in the mutants.

So far, mutations in at least four different genes have been found to give the same class II ampicillin-resistant phenotype. This resistance is observed only on agar plates and is due to a leakage of the β-lactamase through the altered envelope, which creates a zone of ampicillin-destroying enzyme around the cells on the ampicillin plates. This enzyme leakage is enough to save the cells from lysis on agar plates (26).

Mutants sensitive to sodium cholate are rather frequent among the class II mutants (Tables 2 and 3). The sensitivity to cholate may be explained by an increased penetration through the altered envelope to the membrane. Those changes in the cell envelope, which may be due to mutations in several different genes, enable the cholate molecules easily to reach the membrane, which is known to be the target for the action of sodium cholate (26). The *galU* mutants, the *lpsA* mutant, strains G11e5 and G11e6 as well as the G11e1 mutant, which is mutated in a gene located between *bio* and *pyrD* (3), are all class II ampicillin-resistant mutants and sensitive to sodium cholate. The *galU*, *lpsA*, and *lpsB* mutants have changes in the polysaccharide part of their lipopolysaccharide, whereas G11e1 has an intact polysaccharide (3). Taken together, class II ampicillin resistance and cholate sensitivity are strong evidence that *E. coli* cells contain an outer penetration barrier. Leive (14) has shown that ethylenediaminetetraacetate treatment of *E. coli* cells leads to a loss of 30 to 50% of the lipopolysaccharide content of the cells and to a destruction of the penetration barrier (15). The results presented here suggest that lipopolysaccharide is a component of this barrier. However, it must be emphasized that the effect may be more indirect, since a change in one component may lead to steric changes in other parts of the cell envelope. A reduction of the sugar content of the lipopolysaccharide leads to an increased lipophilia which may explain the increased penetration of cholate. The composition of lipopolysaccharide in *galE* mutants is little changed, and they are therefore cholate resistant (Table 9). Class II mutants may be valuable as tools for studying the outer penetration barrier in bacteria.

Similarly, changes in the outer regions of the cell envelope can reduce the ability of the cells to retain their periplasmic enzymes. Thus, class II mutants can be used to study the periplasma of bacteria. Since penicillinases and cholate are very different molecules, it is not surprising that most of the class II mutants are resistant to cholate (Table 3).

Since the bacterial envelope is a complex structure, it is reasonable that the class II phenotype can be obtained by mutations in many different genes.

Other pleiotropic properties of the class II mutants, e.g., sensitivity to phages C21 and φW and inability to accumulate galactose, presumably result from more specific changes of the cell envelope. However, the mutants share these properties with other strains which are not classified as class II ampicillin-resistant mutants. Thus, the ampicillin resistance and the sensitivity to sodium cholate of the class II mutants are secondary properties which show that the cell envelopes are altered in some fashion. As can be concluded from Table 9, the extent by which the composition of the lipopolysaccharide is changed by a mutation will lead to few or many pheno-

typic changes. Since class II ampicillin-resistant mutants are easily isolated, they may be used as a tool for studies of the biosynthesis, composition, and function of the cell envelope.

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