

Mutants Defective in the 33K Outer Membrane Protein of *Salmonella typhimurium*

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Salmonella typhimurium LT2 lines, if phenotypically rough, are fully sensitive to bacteriocin 4-59, produced by *Salmonella canastel* strain SL1712. Bacteriocin-resistant mutants fell into three classes. Those resistant to phage ES18 and to albomycin proved to be mutants of class *chr* (equivalent to *tonB* of *Escherichia coli*); these mutants still adsorb the bacteriocin and so are classified as tolerant. Another class of (incompletely) tolerant mutants was resistant to phage PH51; their envelope fractions lacked the band corresponding to outer membrane protein 34K, known to serve for adsorption of phage PH51. A third class of mutants, which did not adsorb the bacteriocin, was unaltered in sensitivity to phages. Their envelopes lacked the 33K band, indicating absence of the outer membrane protein 33K, considered to correspond to outer membrane protein II* of *E. coli*, which in that species is determined at locus *ompA* (formerly *tolG* or *con*). Phage P22 HT105/1 cotransduced the 33K *S. typhimurium* gene (to be called *ompA*, to accord with *E. coli* usage) with *pyrD*⁺ at about 30% frequency when the donor allele was *ompA*⁺ or one *ompA*, but at only 3 to 11% when the donor allele was another *ompA*. When the donor carried either of two long deletions of the *put* (proline utilization) operon, phage P22 HT105/1 cotransduced *put* (and *ompA*⁺) with *pyrD*⁺ at low frequency. The cotransduction data indicate that *ompA* of *S. typhimurium* is located between *pyrD* and *put*, nearer the former. This corresponds to the map position of *ompA* in *E. coli* K-12.

The major outer membrane (OM) proteins of *Escherichia coli* and *Salmonella* include two distinct classes of proteins in the molecular weight range of 33,000 to 37,000, as measured by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (1, 16). Of these, the larger, 34,000- to 37,000-dalton proteins, called protein I in *E. coli* and proteins 34K, 35K, and 36K in *Salmonella typhimurium*, are resistant to trypsin, have a strong association with peptidoglycan, and function as porins, allowing the relatively free passage of small hydrophilic molecules through the OM (1, 4, 16, 21-23, 35). In *E. coli* the smaller protein, called II*, is trypsin sensitive and heat modifiable in the sense that heating in SDS changes its apparent molecular weight from 28,000 to 33,000 (10). This protein is necessary for recipient function in F-mediated bacterial conjugation, and *con* mutants, recognized as defective in conjugation, lack it (31). In *S. typhimurium* protein 33K is of a similar size and is similarly trypsin sensitive and heat modifiable (1, 24).

Recently, some mutants resistant or tolerant to bacteriophages or bacteriocins or both have

been shown to lack one or another of the major OM proteins. Easy selection methods using OM protein-specific bacteriophages are thus available for isolation of mutants lacking protein I of *E. coli* (7, 8, 29, 33), the 34K and 36K proteins of *Salmonella* (25, 30), or protein II* of *E. coli* (8, 14); however, no method of selecting 33K protein mutants of *Salmonella* has been described previously. We have found that bacteriocin 4-59, produced by *Salmonella canastel* strain 4-59 (12, 13), can be used to select 33K-negative mutants. We here describe the isolation and preliminary characterization of such mutants.

MATERIALS AND METHODS

Most of the *S. typhimurium* strains used (Table 1) are derivatives of subline SL1027 (18, 34) of strain LT2; this subline, which is cured of prophage Fels 2 carried by strain LT2 (34), produces normal amounts of 33K, 34K, and 36K proteins but very little 35K protein (25). A mutant of strain LT2 *pyrD24* found to be galactose negative and sensitive to phage C21 and therefore inferred to be deficient of UDP-galactose epimerase was isolated by selection for resistance to

TABLE 1. *Bacterial strains*

Strain ^a	Description ^b	Origin or reference ^c
Bacteriocin-sensitive strains		
LT2 <i>pyrD24</i>	<i>pyrD24</i>	
SH5014	As SL1027, but <i>ilv-1178 thr-914 his-6116 rfaJ4041</i>	25
SH5551	As SH5014, but <i>ompD106</i>	25
SH6261	As SH5014, but <i>ompD115 ompC336</i>	25
SL1027	<i>metA22 trp-2 H1-b H2-e,n,x</i> "cured of Felix 2" <i>flaA66 rpsL120 xyl-404 metE551</i>	18, 34
SL1657	As SL1027, but <i>hspLT6 hspS29 ilv-452 galE496</i>	
SL1712	<i>S. canastel</i> 4-59, produces bacteriocin 4-59	12, 13
SL1941	<i>pyrD24 galE706</i>	Selected as FO resistant
TR4908	<i>putC900 Δput-521</i>	27; J. Roth ^d
TR4931	<i>putC900 Δput-544</i>	27; J. Roth
SL1224	<i>pyrD24 galE706 put-152</i>	Selected as AC resistant
Mutants selected for resistance to bacteriocin 4-59^e		
SH9097	As SH6261, but <i>ompA204</i>	
SL1906	As SH5014, but <i>ompD199</i>	
SL1908	As SH5014, but <i>chr-55</i>	
SL1909	As SH5014, but <i>ompA201</i>	
SL1912	As SL1657, but <i>chr-56</i>	
SL1915	As SL1657, but <i>ompD197</i>	
SL1917	As SL1657, but <i>ompA202</i>	
SL1918	As SL1657, but <i>ompA203</i>	
SL1225	As SL1941, but <i>ompA205</i>	

^a SH strains are from the collection of P. H. Mäkelä, SL strains are from the collection of B. A. D. Stocker, and TR strains are from the collection of J. Roth, University of Utah.

^b All strains except SL1712 are derivatives of *S. typhimurium* LT2.

^c FO resistant, Resistant to Felix O phage; AC resistant, resistant to azetidine carboxylic acid.

^d Personal communication from J. Roth.

^e Mutants indicated *chr* are in phenotypic class I, those indicated *ompD* are in class II, and those indicated *ompA* are in class III.

Felix O phage (34). Its loss of smooth phenotype caused greatly increased sensitivity to bacteriocin 4-59, the clear zones bordering streaks of the producing strain being about 6 mm wide instead of 1 mm or less.

For tests of bacteriocin sensitivity nutrient agar (Oxoid blood agar base, code CM55) in glass petri dishes was inoculated with streaks of the producing strain, SL1712, which is *S. canastel* 4-59 of Hamon and Péron (13). After overnight incubation at 37°C the bacteria were killed by exposure to chloroform vapor, and a layer of molten soft agar seeded with the strain being tested was added. For simultaneous tests on several strains broth cultures were streaked at right angles to the (chloroform-killed) streak of SL1712, either on the original agar surface or after addition of an uninoculated agar layer. The plates were examined for inhibition zones after overnight incubation. To distinguish nonadsorbing resistant strains from strains able to adsorb the bacteriocin but not killed by it, we used the triple-layer method of Davies and Reeves (7); incubated cross-streaked plates were exposed to chloroform vapor, and a layer of molten soft nutrient agar seeded with a bacteriocin-sensitive indicator, usually strain SL1941 (Table 1), was added. Absence or narrowing of the zone of inhibition of the indicator strain

over the SL1712 streak at the point where it was crossed by the streak of growth of a resistant strain indicated that the latter adsorbed the bacteriocin.

Bacteriocin-resistant mutants, obtained by picking colonies within inhibition zones, were purified by single-colony reisolation and then tested for sensitivity to phages by the drop-on-lawn method (11); the phages used comprised a set of lipopolysaccharide-specific phages (34), phages ES18 and ES18.h1 (17), which utilize an OM protein for adsorption (12), and phages PH51 and PH105, which use proteins 34K and 36K, respectively (25, 30). To score the *put* (proline utilization) character of *pyr*⁺ transductants, they were streaked from broth culture to a solid defined medium with L-proline (2 mg/ml) as the only nitrogen source and to defined medium with L-azetidine-2-carboxylic acid (a growth-inhibiting proline analog) at 50 μg/ml (27). Proline utilization was scored after 1 and 2 days of incubation at 37°C, and growth in the presence of the analog was scored after overnight incubation. (For detection of the ca. 5% *put* clones among the numerous *pyrD*⁺ transductants, colonies were picked directly from selection plates to liquid selective medium in 25-compartment sterile Bertani boxes [autoclavable nylon microculture containers from Elesa, Milan, Italy];

after overnight 37°C incubation of the boxes in 100-by 20-mm glass petri dish bottoms with unglazed porcelain lids, the cultures were inoculated to test plates by using a multiprong replicator.) Spontaneous *put* mutants were obtained by selection for resistance to 25 µg of azetidine carboxylic acid per ml. Their character was confirmed by a test for proline utilization.

For transduction was used an *int* derivative of the high-transducing phage P22 HT105/1 (28). Strains to be used as donors, if *galE*, were grown in medium supplemented with galactose (and glucose to prevent galactose toxicity), to make them phenotypically smooth and therefore sensitive to P22. Transductants were selected by the drop-on-lawn method (11), the selective medium used being supplemented with galactose and glucose if the recipient was deficient in *galE* function.

The OM proteins were characterized by the SDS-polyacrylamide gel electrophoresis method of Laemmli (19), using lysozyme-EDTA cell envelope preparations (25). The gel patterns of mutants were compared with those of known OM protein mutants (25) applied to the same slab gel.

RESULTS

Bacteriocin 4-59, produced by *S. canastel* SL1712, caused inhibition zones 5 mm or more wide of the growth of *galE* strain SL1657 and *rfaJ* strain SH5014. Bacteriocin-resistant mutants were isolated from both strains. Three classes of mutants were obtained from each parent strain, distinguishable by their resistance or sensitivity to certain OM protein phages (Table 2). Class I mutants were resistant to phage ES18 (and to its extended host range variant, ES18.h1 [17]), and class II mutants were resistant to phage PH51; class III mutants, like the parents, were sensitive to all of these phages. In all of these mutants the lipopolysaccharide was unaltered, as indicated by their unaltered pattern of sensitivity to lipopolysaccharide-specific phages.

Class I mutants, which are resistant to phage ES18, are believed to be *chr* mutants (5) and to

correspond to *tonB* mutants of *E. coli*. All class I mutants and several previously reported *chr* mutants of *S. typhimurium* (5) were found to resemble *tonB* mutants of *E. coli* in their resistance to albomycin, an antibiotic active on the parent strains and the other two classes of bacteriocin-resistant mutants. Although not killed by bacteriocin 4-59, all class I mutants tested were able to adsorb it, as shown by the triple-layer test. On the nutrient agar used they produced a reddish pigment, which we believe is a complex of ferric iron with enterochelin (12). The mutations of all class I mutants tested were cotransducible with *trp*⁺, as is *tonB* in *E. coli*. In SDS gels the envelope proteins of class I mutants differed from those of their parents only in showing new or substantially stronger bands in the 86,000- to 88,000-molecular weight range (compare Fig. 1 lanes b and d and lanes g and h). In *E. coli* iron deficiency, which is characteristic of *tonB* mutants, has been shown to result in derepressed levels of OM proteins in this same size class (26).

Class II mutants resistant to bacteriocin 4-59 are resistant to phage PH51 but otherwise unaltered in phage sensitivity. These mutants adsorb the bacteriocin (as shown by triple-layer test) and are, indeed, only partly resistant to it, their growth in soft agar layers being at least thinned over the bacteriocin-producing macrocolony or streak and for up to 1 mm beyond its margin. The phage resistance of class II mutants suggested that they might be mutants lacking OM protein 34K, such as have been isolated by selection for resistance to phage PH51 (25); testing of representative 34K⁻ mutants showed them to be resistant to bacteriocin 4-59. SDS gel electrophoresis of class II bacteriocin-resistant mutants confirmed their resemblance to 34K⁻ mutants isolated as resistant to phage PH51; the samples from class II mutants in Fig. 1 (lanes e and i) indeed have a very faint, if any, band at

TABLE 2. Properties of mutants selected as resistant to bacteriocin 4-59^a

Class	Bacteriocin 4-59 ^b		Sensitivity to phage ^c		Albomycin sensitivity	Pigment production	Envelope protein changes ^d
	Sensitivity	Adsorption	PH51	ES18			
Parents	S	+	S	S	S	-	NA
I	R	+	S	R	R	+	86K-88K up
II	r	+	R	S	S	-	34K negative
III	R	-	S	S	S	-	33K negative

^a The parent strains (SH5014, SL1657, and SL1941) are all phenotypically rough, because of *rfaJ* or *galE* mutations.

^b S, Fully sensitive; R, completely resistant; r, almost completely resistant. Adsorption was tested by the triple-layer method: +, adsorption; -, no adsorption.

^c Phage sensitivities were tested by application of a drop or loopful of lysate, usually at about 10⁸ plaque-forming units per ml, to streak inoculum. Phage ES18.h1 was used for tests on strains not cured of Fels 2 (17).

^d NA, Not applicable; up refers to increases in bands of proteins in the indicated molecular mass range; negative means that the band of protein of indicated molecular mass was absent.

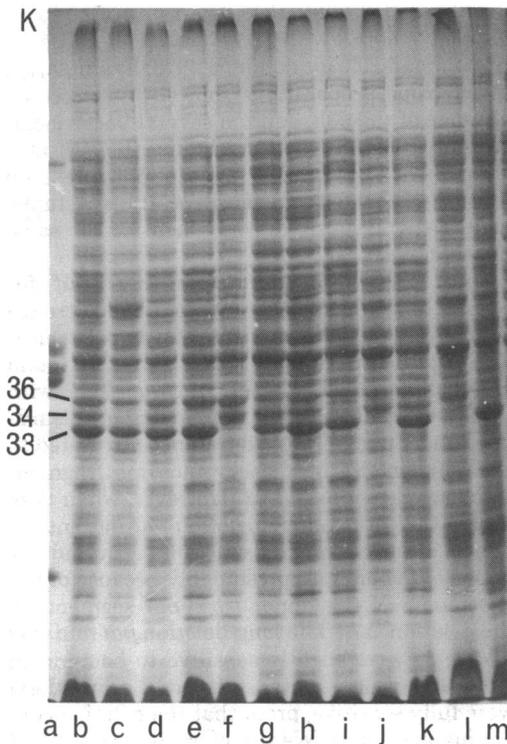


FIG. 1. SDS-polyacrylamide (10%) gel protein pattern of lysozyme-EDTA envelopes. The 34K and 36K OM protein bands are marked as identified by the previously described mutants SH5551 (lane c) and SH6261 (lane m). Lane a, Molecular weight standards: from top to bottom, phosphorylase A (94,000), ovalbumin (43,000), alcohol dehydrogenase (37,000), trypsin (23,000), myoglobin (17,200), and lysozyme (14,000); the last two ran with the front. Lane b, Parent SH5014; lane c, its 34K⁻ mutant SH5551. Lanes d through f, Bacteriocin-resistant mutants of SH5014: lane d, SL1908 (class I, tonB-like); lane e, SL1906 (class II, 34K⁻); and lane f, SL1909 (class III, 33K⁻). Lane g, Parent SL1657. Lanes h through j, Bacteriocin-resistant mutants of SL1657: lane h, SL1912 (class I, tonB-like); lane i, SL1915 (class II, 34K⁻); and lane j, SL1917 (class III, 33K⁻). Lane k, Parent SH5014. Lane l, Bacteriocin-resistant, 33K⁻ mutant SH9097 of SH6261. Lane m, SH6261, a 34K⁻ 36K⁻ derivative of SH5014.

the 34K position and look like the standard 34K⁻ mutant SH5551 (lane c). (Note that mutant SH5551 has an extra band of approximate molecular weight 52,000; this feature is not connected with its PH51 resistance or its lack of 34K protein, as shown by tests of other 34K⁻ mutants [32] and of 34K⁺ recombinant derivatives of SH5551 [Nurminen and Mäkelä, unpublished data].)

Class III mutants, resistant to bacteriocin 4-59 but sensitive to phages ES18 and PH51, did not

adsorb the bacteriocin, as shown by the results of triple-layer tests. Presumably, therefore, they lack the bacteriocin receptor substance in their OM. The SDS gels of the envelope proteins of two class III mutants, SL1909 and SL1917 (Fig. 1, lanes f and j), showed correspondingly a very marked reduction of the 33K band. It is not clear whether the faint band seen in the mutant samples at the approximate position of the 33K protein in the parent represents residual 33K protein or another peptide which in the parent was hidden by the strong 33K band. One class III mutant, strain SL1917 (Fig. 1, lane j), also showed a marked reduction or absence of bands having approximate molecular weights of 60,000, 25,000, and 22,000. This feature was also seen in several other class III mutants (data not shown).

We also selected mutants resistant to bacteriocin 4-59 from parent strain SH6261, which almost entirely lacks protein 35K (because it is derived from the SL1027 line), protein 34K (because of mutation *ompD115*, secured by selection for resistance to PH51), and protein 36K (because of mutation *ompC336*, obtained by selection for resistance to phage PH105 [25]). Despite the mutation causing the loss of 34K protein, strain SH6261 was still partly sensitive to bacteriocin 4-59, so that resistant mutants could be detected as papillae in the area of thinned growth overlying the macrocolony of the bacteriocinogenic strain. The envelope proteins of strain SH6261 can be seen Fig. 1 (lane m); the 33K band is strong. Its bacteriocin-resistant derivative, SH9097 (lane l), like the other 33K⁻ mutants, has a faint band in the approximate 33K position; it has more protein than the parent at the 34K position and also, most pronouncedly, at the 35K position, but the total amount of material in the 33K to 36K area is less than in any of the other mutants in the gel.

In *E. coli* K-12 gene *tolG*, now called *ompA* and thought to be the structural gene for protein II*, was mapped at ca. 21.5 min, near *pyrD* and between it and *fabA* (2, 8). To test for a similar location of the 33K gene in *S. typhimurium*, we isolated a *galeE* (therefore phenotypically rough and fully bacteriocin-sensitive) mutant from LT2 *pyrD24*. Phage P22 HT105/1 *int* lysates of two class III mutants, SL1917 and SL1918, which were both proven to be 33K⁻ and therefore designated *ompA* (to accord with *E. coli* usage), were used to evoke *pyr*⁺ transductants from SL1941 (=LT2 *pyrD galeE*). Such transductants, after single-colony reisolation or after growth in liquid defined medium without uracil, were tested for sensitivity to the bacteriocin. In the first such experiment (Table 3, crosses 1 and 2), 1 of 28 *pyrD*⁺ transductant clones evoked by

phage grown on SL1917 (*ompA202*) was found to include both bacteriocin-sensitive and bacteriocin-resistant components, as was 1 of 24 transductants evoked by phage grown on SL1918 (*ompA203*). If these mixed clones, as well as bacteriocin-resistant clones, are counted as hav-

ing the donor *omp* allele, the rates of cotransduction of *ompA* with *pyrD*⁺ were 11% for donor SL1917 and 29% for donor SL1918. Examination of envelope preparations showed the expected absence of the 33K band in several bacteriocin-resistant transductants, including the resistant component of one mixed clone, and its presence in several bacteriocin-sensitive transductants, including the sensitive component of the same mixed clone.

In *S. typhimurium* an operon called *put* (for uptake of proline and its utilization as nitrogen source) is located near *pyrD* and is between it and *fabA* (27). Ratzkin and Roth (27) found about 9% cotransduction of point *put* alleles with *pyrD*⁺ by phage P1, but no detectable cotransduction by phage P22, a difference attributed to the smaller size of the chromosome fragment transduced by phage P22. However, when they used either of two long-deletion *put* mutants as donor, a few *pyrD*⁺ transductants acquired the donor *put* character, presumably because of the shortening of the donor *pyrD-put* segment by the deletion (27). The long-deletion *put* mutants (Table 1) were slightly sensitive to bacteriocin 4-59, and rough mutants isolated from them were fully sensitive, proof that the deletions did not involve *ompA*. We therefore used phage P22 HT105/1 *int* grown on the two *put* long-deletion mutants TR4908 and TR4931 to obtain *pyrD*⁺ transductants from a bacteriocin-resistant mutant of SL1941 (=LT2 *pyrD gale*); this mutant was shown to be 33K⁻ by a test of an envelope preparation. Co-transduction of donor *ompA*⁺ with *pyrD*⁺ was observed at frequencies of 36 and 32% (Table 4, crosses 5 and 6). However, only 1 of 50 *pyrD*⁺ transductants acquired the donor *Put*⁻ character (and also donor *ompA*⁺), and attempts to select *Put*⁻ transductants on defined medium with proline analog were unsuccessful because of large numbers of analog-rec-

TABLE 3. Cotransduction of *ompA* with *pyrD*⁺ by P22 HT105/1 *int*^a

Cross no.	Recipient ^b	Donor ^c	No. of <i>pyrD</i> ⁺ transductant clones of the indicated genotype classes (donor alleles):		
			<i>pyrD</i> ⁺	<i>pyrD</i> ⁺ <i>ompA</i>	<i>pyrD</i> ⁺ and <i>pyrD</i> ⁺ <i>ompA</i> (mixed)
1	SL1941	SL1917	25	2	1
2	SL1941	SL1918	17	6	1
3	SL1224	SL1917	29 ^d	1 ^d	0
4	SL1224	SL1918	17 ^d	13 ^d	0
9 ^e	SL1224	SL1917	32	2	
10 ^e	SL1224	SL1918	34	11	

^a P22 HT105/1 *int* grown on the indicated strain as donor was used to evoke *pyr*⁺ transductants from the indicated recipient strains. (The lysate of strain SL1917 used in crosses 3 and 9 was not the same as that used in cross 1.) The *ompA* character was inferred from bacteriocin sensitivity of single-colony reisolates of *pyr*⁺ transductants.

^b SL1941 is LT2 *pyrD24 gale706*; SL1224 is a *put-152* mutant of SL1941.

^c SL1917 and SL1918 are 33K⁻ mutants of SL1657 (*ompA202* and *ompA203*, respectively).

^d None of the transductants selected as *pyrD*⁺ had donor *put*⁺ character.

^e In crosses 9 and 10 all *Pyr*⁺ transductant colonies detected in several drop areas after incubation for several days were tested for bacteriocin sensitivity; transductant clones including both *pyrD*⁺ *ompA* and *pyrD*⁺ *ompA*⁺ components would have been scored as *ompA*.

TABLE 4. Cotransduction of *ompA*⁺ and *put* with *pyrD*⁺ by P22 HT105/1 *int*^a

Cross no.	Recipient ^b	Donor ^c	No. of <i>pyrD</i> ⁺ transductant clones with the following transductant classes (donor alleles):				
			<i>pyrD</i> ⁺	<i>pyrD</i> ⁺ <i>ompA</i> ⁺	<i>pyrD</i> ⁺ <i>ompA</i> ⁺ <i>put</i>	<i>pyrD</i> ⁺ <i>put</i>	<i>pyrD</i> ⁺ (?) ^d
5	SL1225	TR4908	16	8	1	0	0
6	SL1225	TR4931	17	8	0	0	0
7	SL1225	TR4908	7	3	4	1	84
8	SL1225	TR4931	7	3	6	0	59

^a P22 HT105/1 grown on the indicated *put* deletion strain as donor was used to evoke *pyrD*⁺ transductants from *ompA* recipients. The *omp* character of purified transductants was inferred from bacteriocin sensitivity. In crosses 7 and 8 nonpurified transductant clones were screened to detect *put* transductants.

^b SL1225 is *pyrD24 gale706 ompA205*.

^c TR4908 is Δ *put-521*; TR4931 is Δ *put-544*.

^d These transductants, found not to have donor *put*, were not tested for the *omp* character.

sistant mutants. The crosses were therefore repeated (Table 4, crosses 7 and 8), and larger numbers of Pyr⁺ transductants were screened for the Put character (see above). All transductants confirmed as *put* after single-colony reisolation and a sample of similarly purified transductants found to be *put*⁺ were scored for bacteriocin sensitivity; in each cross the donor *ompA*† allele was present in a higher proportion in the transductants with donor *put* allele than in those with recipient *put*⁺ allele (for Δ*put*-521 donor, 4 of 5 *put* transductants were *ompA*⁺, versus 3 of 10 *put*; for Δ*put*-524 donor, 6 of 6 *put* were *ompA*⁺, versus 3 of 10 *put*⁺). In another experiment (Table 3, crosses 3 and 4), we used as the recipient a *put* mutant, SL1224, which was derived from SL1941 (=LT2 *pyrD galE*) by selection for resistance to azetidine carboxylic acid, and as donors the two *ompA* mutants used previously (SL1917 and SL1918). Selection for ability to utilize proline yielded *put*⁺ transductants, all 43 of which, however, retained the recipient *pyrD* and *ompA*⁺ alleles. Similarly, all transductants selected for acquisition of donor *pyrD*⁺ retained the recipient *put* allele. The failure of phage P22 to cotransduce *put* and *pyrD* is as expected for a donor in which the distance between these loci has not been shortened by deletion (27).

In the above-mentioned crosses some *pyrD*⁺ transductants had the donor bacteriocin resistance, i.e. 33K⁻, character; the representation of donor *ompA* was 3% when the donor was SL1917 (*ompA202*) and 42% when the donor was SL1918 (*ompA203*) (Table 3, crosses 3 and 4). We thought that in this and the earlier transduction experiment with SL1917 as donor (Table 3, cross 1) we might have inadvertently chosen earlier appearing or larger transductant colonies for testing of bacteriocin sensitivity; if *ompA202* caused slow growth or small colony size on defined medium, this would result in an apparent rate of cotransduction of *ompA* with *pyrD*⁺ lower than the true rate. We therefore performed a further experiment with SL1224 (*pyrD galE put*) as recipient and used various dilutions of P22 HT105/1 lysates of SL1917 (*ompA202*) and SL1918 (*ompA203*) to evoke *pyrD*⁺ transductants. The selection plates were incubated for several days at 37°C; then all Pyr⁺ colonies seen in several drop areas were picked and transferred to liquid defined medium without uracil, and the resulting cultures were tested for bacteriocin sensitivity (Table 3, crosses 9 and 10). As in the earlier experiments, the representation of *ompA202* of donor SL1917 (2 of 34 or 6%) was significantly lower than that of *ompA203* of SL1918 (11 of 45 or 25%).

DISCUSSION

The bacteriocin produced by *S. canastel* SL1712 does not act on *E. coli* K-12 or B but is active on *S. typhimurium* strain LT2; however, like many colicins, it is much less active on smooth than on rough strains. All colicins which have been investigated in this respect have been found to adsorb to proteins of the OM, and it was therefore surmised that bacteriocin 4-59 adsorbed to an OM protein differing in composition from any corresponding protein of *E. coli*. Selection for bacteriocin resistance yielded three classes of mutants, each class having characteristic alteration(s) in OM composition. Class I mutants were inferred from their phenotypes and from the locations of their genes to be of type *chr* (5, 12), corresponding to *tonB* in *E. coli* (2, 3, 7). The increase in intensity of bands representing certain large OM proteins in envelope preparations of class I mutants presumably reflects their expected defective assimilation of iron. Bacteriocin-resistant mutants of class II were resistant to phage PH51, and their envelopes lacked the band corresponding to protein 34K, which is known to adsorb this phage. As these mutants still adsorbed bacteriocin 4-59 (and were, indeed, slightly sensitive to it), it appears that OM protein 34K is needed for some postadsorption stage of action of this bacteriocin, just as OM protein 1a of *E. coli* is needed for action of colicins A, L, and K (8).

Bacteriocin-selected mutants of class III were not altered in phage sensitivity and had no conspicuous phenotypic changes, but SDS gel electrophoresis of their envelopes showed them to be deficient in the 33K band, a protein which (see above) corresponds in various properties to protein II* of *E. coli* K-12. *E. coli* mutants lacking protein II* can be obtained by selection for resistance to phages thought to adsorb to this protein, i.e. phage K3 (20) or TuII* (14), but no phage specific for 33K⁺ *S. typhimurium* has been discovered. The use of the triple-layer method showed that the 33K⁻ mutants failed to adsorb bacteriocin 4-59, which presumably accounts for their bacteriocin resistance; in contrast, *E. coli* K-12 mutants lacking protein II* still adsorb colicin L, although they are unaffected by it (8). In *E. coli* K-12, mutations causing loss (or alteration) of protein II* map at a locus now termed *ompA*, which is located at ca. 21.5 min, between *pyrD* and *fabA* (2, 8). We observed cotransduction of the gene determining wild-type bacteriocin sensitivity (i.e., *ompA*⁺ of *S. typhimurium*) with *pyrD*⁺ by phage P22 at frequencies of 30 to 36% (Table 4) and cotransduction of two mutant *ompA* alleles with *pyrD*⁺

at frequencies of 29, 42, and 25% for the *ompA203* donor SL1918 but at only 11, 3, and 6% for the *ompA202* donor SL1917 (Table 3). In *S. typhimurium* the *put* operon is close to *pyrD* and is between it and *pyrC* (27). Analogy with the gene order in *E. coli*, *-pyrD-ompA-fab-pyrC-* (2), therefore predicts the order *-pyrD-(ompA, put)-pyrC-* in *S. typhimurium*. The *pyrD-put* interval is too long for phage P22 to cotransduce *put* with *pyrD*⁺ except when the distance in the donor has been reduced by a long deletion (27). Our observation of 32% cotransduction of *ompA*⁺ with *pyrD*⁺ by phage P22 (Table 4) therefore shows the order in *S. typhimurium* to be *-pyrD-ompA-put-*. In crosses with the long-deletion *put* mutants as donors (Table 4, crosses 7 and 8), the higher representation of donor *ompA*⁺ among the few *pyrD*⁺ transductants with donor *put* confirms this order.

There is good evidence that *ompA* is the structural gene for protein II* in *E. coli* (6, 15). The frequencies of cotransduction of mutation *ompA203* with *pyrD*⁺ in three experiments (29, 42, and 43%; Table 3) were about the same as the rates of cotransduction of *ompA*⁺ with *pyrD*⁺ to the *pyrD ompA205* recipient in two experiments (36 and 32%; Table 4). It is therefore reasonable to assume that *ompA203* and *ompA205* affect the same gene, perhaps the structural gene for protein 33K. In contrast, the frequencies of cotransduction with *pyrD*⁺ for *ompA202* of strain SL1917 were 11 and 3% in our first two experiments, in which two different P22 HT105/1 *int* lysates of SL1917 were used (Table 3), and 6% in another experiment, in which we took precautions to avoid choosing larger or earlier appearing *Pyr*⁺ transductant colonies for tests of bacteriocin sensitivity. The difference between the frequency of cotransduction of *ompA202* and the frequencies of transduction of the other two alleles is greater than would be expected for different point mutations in a single protein-specifying gene. Perhaps there are two genes located between *put* and *pyrD* concerned with production of protein 33K, one the structural gene for this protein and the other of unknown function, conceivably the operator-promoter region of an operon which includes the structural gene. Another possibility would be that mutation *ompA202* of strain SL1917 is a chromosomal anomaly, e.g., an insertion, which reduces the probability of incorporation of the affected gene into the chromosome of the transductional recipient.

Alterations in envelope morphology caused by *ompA* mutations are described by Lounatmaa in the accompanying paper (19a). In *E. coli* K-12 an *ompA* mutation causing loss of protein II*

results also in loss or diminution in ability to serve as female parent in conjugational crosses mediated by the F factor (31); in preliminary experiments we observed a similar decrease in ability to accept an F'*gal*⁺ factor in a 33K⁻ derived from strain SL1657. *E. coli* K-12 mutants now designated *ompA* have been reported as more sensitive than wild-type strains to certain antibiotics and other antibacterials (8, 9). We do not yet have adequate data on possible similar effects of *ompA* mutation in *S. typhimurium*.

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