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Iron Transport in *Salmonella typhimurium* LT-2: Prevention, by Ferrichrome, of Adsorption of Bacteriophages ES18 and ES18.h1 to a Common Cell Envelope Receptor

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Ferrichrome prevents adsorption of phages ES18 and ES18.h1 to cells of *Salmonella typhimurium*. Studies of albomycin-resistant mutants defective in both ferrichrome utilization and ES18.h1 adsorption suggest a *sid* gene may specify a receptor for both.

Escherichia coli has a number of outer membrane receptor proteins which have been shown to be involved in both transport of specific nutrients and attachment of phages and colicins. Thus the receptor for T1, T5, and ϕ80 and colicin M is required for ferrichrome transport (4, 7, 10), the receptor for colicin B is involved in enterobactin transport (2, 11), the receptor for λ is involved in maltose transport (9), and the receptor for colicins E1 and E3 and phage BF23 is involved in transport of vitamin B_{12} (1).

Receptors involved in transport would be expected to occur in the outer membrane of *Salmonella typhimurium*. A common receptor for the E colicins and phage BF23 which may play a role in B_{12} transport has been reported (3). We have obtained preliminary evidence that the receptor for phages ES18 and ES18.h1 is required for efficient ferrichrome utilization by *S. typhimurium* LT-2 (M. Luckey and J. B. Neilands, Abstr. Pacific Slope Biochem. Conf. 1975, vol. 17, p. 53).

Mutants of *S. typhimurium* that grow poorly on ferrichrome have been isolated by resistance to albomycin (6). In a survey of *Salmonella* species Stocker noticed a correlation between sensitivity to albomycin and phage ES18 (B. A. D. Stocker, personal communication). The latter attacks smooth and rough strains of *S. typhimurium* not lysogenic for Fels2; ES18.h1 is a host-range mutant that can propagate on strains carrying Fels2 (5).

Phages ES18 and ES18.h1 and an *S. typhimurium* strain sensitive to ES18, SL1027, were kindly provided by B. A. D. Stocker. *S. typhimurium* blocked in enterobactin biosynthesis, *enb-7*, and the albomycin-resistant mutants *sidC39* (TA2738) and *sidF53* (TA2752) have been described previously (6, 8). Additionally, the albomycin-resistant mutant *sid-68* (TA2767) also grows poorly on ferrichrome and is 35% cotransducible with *panC*. Although the factors regulating the nutritional response to siderophores are not yet completely understood, these three mutants appear to be similar in their diminished utilization of ferrichrome, inability to grow on albomycin as an iron source, and normal ability to use structurally different siderophores such as rhodotorulic acid and schizokinen.

We report that micromolar concentrations of ferrichrome protect *S. typhimurium* in vivo from both ES18 and ES18.h1. Ferrichrome does not inactivate the phages irreversibly, since preincubation in ferrichrome both at 4°C overnight and at 37°C for 4 h had no effect on the plaque-forming ability of the phages. Ferrichrome at a concentration of 100 μM prevented the adsorption of both ES18 and ES18.h1 to the *enb-7* mutant of *S. typhimurium* (Table 1). Although ES18 and ES18.h1 adsorb very slowly, from 65 to 80% adsorption is obtained in 30 min at 37°C in the absence of ferrichrome.

Ten classes of *sid* mutants (6) were tested for sensitivity to ES18.h1. All but four were fully sensitive to the phage and protected from it by 250 μM ferrichrome. Three *sid* mutants were completely resistant to ES18.h1 and adsorbed the phage weakly (Table 2). All three carry *sid* mutations that cotransduce with *panC* (6) and may be affecting a cistron analogous to *tonA* in *E. coli*.

These results suggest a model wherein a common site on the cell envelope binds ferrichrome as well as phages ES18 and ES18.h1 and a protein at this site, specified by a *sid* gene, is involved in ferrichrome transport in *S. typhimurium*.

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TABLE 1. *Effect of ferrichrome on adsorption of ES18 and ES18.h1 by enb-7*[a]

Phage	Ferrichrome (μM)	Adsorption (%)
ES18	0	64
	100	0
ES18.h1	0	80
	100	<10

[a] Adsorption was assayed as described previously (8), except cells and phage were preincubated for 30 min at 37°C, and media used were tryptone top agar (8) and nutrient agar plates. ES18 phage were adsorbed to *enb-7* and assayed with SL1027. ES18.h1 phage were adsorbed to *enb-7* and assayed with *enb-7*.

TABLE 2. *Adsorption of ES18.h1 by resistant mutants*[a]

Cells	PFU/plate[b]	Adsorption (%)
None	112, 113, 131	
enb-7	34, 31, 44	70
sid-68	119, 106, 97	<10
sidC39	75, 90, 89	30
sidF53	81, 78, 88	30

[a] Adsorption was measured as described in Table 1.

[b] PFU, Plaque-forming units.

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