Absence of Immunity to Superinfection in Spheroplasts of a Lysogen

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Penicillin-induced spheroplasts of *Salmonella typhimurium* strain LT2 lysogenic for bacteriophage P22, appear to lose immunity to superinfection by homologous phage.

Ray and Burma (4) reported that penicillin-induced spheroplasts of *Salmonella typhimurium* strain LT2 adsorb bacteriophage P22 normally. The infected spheroplasts show single-step growth curves similar to those of infected whole cells, except that the burst sizes are lower. However, induction of phage production in spheroplasts of cells lysogenic for a temperature inducible prophage (3) could not be demonstrated. When examining some properties of the spheroplasts, we noticed a very curious consequence of the penicillin treatment. Spheroplasts of LT2 lysogens lose immunity to superinfection by homologous phage.

Spheroplasts of lysogenic and nonlysogenic cells were produced by the method of Ray and Burma (4) as adapted from Spizizen (5). Cells from slant cultures were inoculated in Pennassay broth (1.5 g of beef extract, 1.5 g of yeast extract, 5 g of peptone, 3.5 g of NaCl, 3.68 g of K_2HPO_4 , 132 g of KH_2PO_4 , and 1 g of dextrose in 1 liter of distilled water) and incubated overnight. The overnight culture (3 ml) was added to 10 ml of Pennassay broth containing 20% sucrose, 0.2% $MgSO_4 \cdot 7H_2O$, and 1×10^8 units of penicillin G per ml. The diluted culture was incubated for 2 hr at 35°C, during which time the optical density doubled. The cells became round as seen under phase-contrast microscopy and were osmotically very fragile. The cultures were cooled, washed twice by centrifugation with equal volumes of cold 20% sucrose containing 0.002 M tris (hydroxymethyl) aminomethane-hydrochloride (pH 7.0), and then suspended in Pennassay broth containing 20% sucrose. The spheroplast suspensions were assayed for colony formers by platings on tryptone agar plates. No more than 1% of the treated cells produced colonies. The whole cell controls were treated in the same way

as spheroplasts except that no penicillin was added.

All infections and dilutions were carried out in Pennassay broth containing 20% sucrose.

A comparison was made of infections of sensitive and lysogenic cells as whole cells and spheroplasts infected with virulent and nonvirulent P22 phages. The virulent mutant, vir-3, which plates on both sensitive and lysogenic whole cells was isolated by Morley Bronson. The nonvirulent phage used was the c_1 mutant of P22 (1, 2) which grows on sensitive cells only. Multiplicities of 5 were used in all infections.

Infections of whole cells served as controls for those in spheroplasts. Both phages replicated in the sensitive host, producing almost identical yields (Table 1, lines Ia and b). As expected, the virulent phage replicated in the lysogenic host (Table 1, line Id). In contrast, no phage progeny were produced in the c_1 infection of the lysogen (Table 1, line Ic). The lysogenic cells survived at the multiplicity used. That is, c_1 phage is sensitive to the immunity repressor. The small number of phage found were derived from the prophage by spontaneous induction.

Spheroplasts of sensitive cells supported the replication of the virulent and nonvirulent phages equally well (Table 1, lines IIa and b). All the spheroplasts were infected and each gave rise to progeny phages, but the burst sizes were considerably less than those for whole cells. These data confirm the report of Ray and Burma (4). In sharp contrast to the results with whole lysogens, both the virulent and nonvirulent infections produced progeny phages in the lysogenic spheroplasts (Table 1, lines IIc and d). The progeny phages were all of the superinfecting type.

These data imply that the immunity to superinfection normally exhibited by lysogens is inoperative in spheroplasts. Whether this is due to inactivation of repressor, to interference with

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TABLE 1. *Production of phage P22 in whole cells and spheroplasts of Salmonella typhimurium LT2*

Host	Superinfecting phage	Infected complexes	Phage yield	Phage yield/cell
I. Whole cells				
a. LT2	<i>c</i> ₁	3.4×10^8	6.5×10^{10}	191
b. LT2	vir-3	3.8×10^8	7.6×10^{10}	200
c. LT(P22)	<i>c</i> ₁	— ^a	2.0×10^{5b}	
d. LT(P22)	vir-3	4.4×10^8	2.1×10^{10}	48
II. Spheroplasts				
a. LT2	<i>c</i> ₁	4.4×10^8	8.5×10^9	19
b. LT2	vir-3	4.6×10^8	9.2×10^9	20
c. LT(P22)	<i>c</i> ₁	3.9×10^8	4.0×10^9	10
d. LT(P22)	vir-3	3.9×10^8	5.1×10^9	13

^a The lysogenic cells survived the infection.

^b These phages are derived from the prophage rather than the superinfecting phage.

repressor action, or to leakage of repressor from the cell, has not been determined. The demonstration that lysogenic spheroplasts can support phage development suggests that the inability to induce phage in these spheroplasts (4) is due to the failure of an earlier step, perhaps excision of the prophage. Penicillin inhibits cell wall biosynthesis (6, 7). These data again emphasize the importance of the integrity of the cell surface to phage-host interactions.

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