Escherichia coli: Strains That Excrete an Inhibitor of Colicin B

Abstract. *Mutants* exb *of* Escherichia coli *are insensitive to colicin B and excrete into their culture media a material that inhibits colicin activity and that appears to be the bacterial lipopolysaccharide.*

In the process of testing *Echerichia coli* mutants resistant to colicin B by the method of Hill and Holland (*1*) we discovered a class of mutants called *exb* that excrete into the medium a substance which inhibits colicin B action and appears to be, or contain, the bacterial lipopolysaccharide.

In the Hill-Holland method, mutants insensitive to colicin are grown in patches on the surface of agar plates containing colicin; the plates are then overlaid with bacteria sensitive to colicin in a thin agar layer. Mutants that can adsorb the colicin prevent it from reaching the sensitive cells, which therefore grow above them. The class of mutants we describe here were completely insensitive to colicin B and, in the Hill-Holland test, generated large halos of sensitive bacterial growth above and around each patch (Fig. 1). Similar halos of protection were obtained if the patches of mutant cells insensitive to colicin were removed before the overlay of sensitive cells was added.

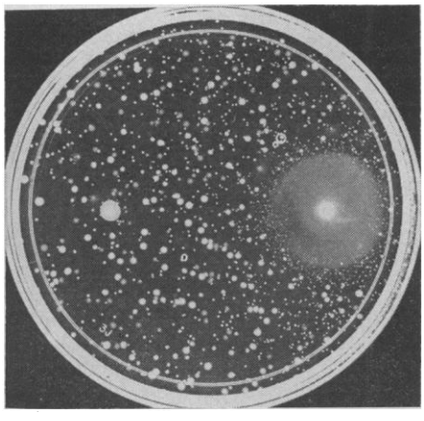

Fig. 1. Production of a "halo" by an *exb* mutant. (Left), *S. typhimurium* LT-2 cells, which are colicin B resistant, do not protect; (right), *E. coli* K-12 *exb* cells have excreted an inhibitor permitting growth of sensitive cells.

The presence of protecting material in culture fluids of the mutant strains could be demonstrated and quantitated by experiments in liquid medium (Table 1). Some of these excreting mutants are also insensitive to colicins Ia and Ib, and their culture fluids inhibit the action of these colicins, but not that of colicins A, E1, E2, E3, or K. Some of the mutants are insensitive also to colicin V, but inhibition of this colicin has not been demonstrated.

Protection of sensitive cells was not increased by prior incubation of the colicin with the protecting material before addition of the cells. The protecting material proved to be filtrable, nondialyzable, stable to heat (10 minutes in boiling water), and insensitive to trypsin and ribonuclease. These properties suggested that the active component was not an enzyme that inactivated colicin B, but a substance that competed with cells for the colicin, as free receptors would. Lipopolysaccharide (LPS) was the most likely candidate. In fact, LPS extracted (*2*) from cells of a variety of *E. coli* K-12 derivatives proved to have inactivating power for colicin B (Table 1).

Mutants of the excreter type were isolated also from *E. coli* B and the colitose-containing smooth strain 0111: B4, and also from mutants of K-12 or B defective in uridine-diphosphoglucose pyrophosphorylase (*3*), whose lipopolysaccharide contains only the innermost polysaccharide core (*4*). The LPS extracted from *E. coli* K-12, B, or 0111: B4, or from their excreter mutants, inhibited colicin B.

We do not know whether in *E. coli* cells LPS is the actual surface receptor for colicin B or not. We have not yet obtained any mutants of *E. coli* resistant to colicin B whose LPS did not inactivate the colicin. Since, however, this colicin is adsorbed very slowly, our current adsorption measurements are not adequate to permit us to decide whether all the mutants insensitive to colicin B which we tested are of the *tol* class [which adsorbs a colicin but does not respond to it (*5*)]. Even if LPS were the receptor for colicin B, it is not excluded that some nonadsorbing mutants might, upon extraction, yield an LPS capable of combining with colicin.

Table 1. Protection of sensitive bacteria against colicin B by filtrates and by LPS preparations. The filtrates were centrifugal supernatants of nutrient broth cultures in the log phase of growth, filtered through Millipore membranes (0.45 μ). The LPS preparations of known content were used at about 1 mg/ml; the others were diluted to correspond to LPS from about 10^{11} cells in the test mixture. Each mixture contained equal volumes of test material and of colicin preparation (or of diluent in the controls). The indicator cells for colicin activity, from a growing culture of *E. coli* B resistant to streptomycin, were added at appropriate concentration. The mixtures were incubated at 37°C for 15 minutes; then 0.1 ml samples were transferred to 2.5 ml of melted soft nutrient agar and poured on nutrient agar plates containing streptomycin sulfate (200 μg/ml). Positive protective activity in test materials is evidenced by colony counts significantly higher than those in control tubes. The absolute concentrations of colicin B used differed slightly from experiment to experiment.

Test material	Number of colonies at relative concentration of colicin B		
	2×	1×	0
E. coli B filtrate	1	2	379
E. coli Bexb1 filtrate	32	181	318
E. coli B/4$_0$ filtrate*	0	14	328
E. coli B/4$_0$ *exb7* filtrate	92	186	382
Control	4	13	292
E. coli B LPS	177	258	383
E. coli K-12 LPS	204	286	363
E. coli K-12 LPS (1:10)	135	224	
Control	9	113	312
E. coli 0111:B4 LPS	493	530	501
Control	46	146	481

* Mutant of *E. coli* B defective in uridine diphosphoglucose pyrophosphorylase (*3*).

SONIA K. GUTERMAN
S. E. LURIA
Department of Biology, Massachusetts Institute of Technology, Cambridge

References and Notes

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