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Mutants of *Escherichia coli* Sensitive to Antibiotics

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Mutants of *Escherichia coli* sensitive to the antibiotic synergistin A, an inhibitor of protein synthesis, were isolated. These mutants were pleiotropic, being also sensitive to a large number of unrelated antibiotics and to lysis by detergents. These pleiotropic responses indicated that the mutations affected cell wall or membrane synthesis. Consequently, selection for antibiotic-sensitive mutants constitutes a useful means for isolating cell wall or membrane mutants.

Wild-type *Eschierichia coli* is naturally resistant to a wide variety of antibiotics which can inhibit growth of gram-positive organisms. However, this resistance is, in many cases, due to the inability of the drugs to enter the cell. In support of this idea are the observations that ethylenediaminetetraacetate-treated *E. coli* cells and cell-free extracts can be inhibited by antibiotics which are ineffective in the intact cell (9) and by the isolation of mutants sensitive to antibiotics at concentrations which normally do not inhibit growth of the parent (2, 8, 10, 12).

During the course of a study on the effect of a variety of antibiotics on protein synthesis in intact *E. coli* cells, it became necessary to isolate mutants sensitive to the drugs. This communication describes the properties of two of these mutants. We show that these mutants are pleiotropic, being sensitive to a large number of unrelated antibiotics and certain detergents. Since these mutants apparently have an altered cell wall or membrane, the study points to a possible way of isolating cell wall or membrane mutants.

MATERIALS AND METHODS

Bacterial strains. Several independent isolates derived from *E. coli* W1895 that were sensitive to synergistin A (PA114A) were isolated by nitrosoguanidine treatment (1), and subsequent replication of colonies on mineral salts-glucose medium, with and without 100 μg of synergistin A per ml. Colonies which failed to grow on media containing the antibiotic but grew on the unsupplemented medium were picked and purified. Strain W1895, the parent strain, can grow normally in the presence of this concentration of antibiotic. Wild-type strain W1895 and the two sensitive mutants studied, H101 and H135, require methionine for growth, are gram-negative rods, are able to ferment lactose, are susceptible to phage f2, and are Hfr.

Media and growth of bacteria. The bacteria were grown either on a mineral salts medium (4) supplemented with 0.5% glucose and 100 μg of methionine per ml or on a mineral salts medium supplemented with 0.3% Casamino Acids (3). The mutants grew slightly less rapidly than the parent strain (which had a mass doubling time of 65 min in mineral salts-glucose medium) but the differences, although consistent, were small. Suspensions of the cells were grown at 37 C with vigorous shaking, and exponentially growing cells were used at a concentration of about 5×10^8 cells/ml.

The media containing agar are listed in each individual experiment.

Antibiotics. The antibiotics used were gifts from many individuals who are listed in the acknowledgments.

Measurement of incorporation of radioactivity into ribonucleic acid (RNA) and protein. Uracil-*2*-^{14}C (56.3 mCi/mmole) and L-leucine-*1*-^{14}C (30.5 mCi/mmole) were purchased from New England Nuclear Corp., Boston, Mass. Incorporation of ^{14}C-leucine and ^{14}C-uracil into trichloroacetic acid-insoluble material was used as an index of protein and RNA synthesis, respectively. The details of the method were previously described (3).

RESULTS

Sensitivity to antibiotics and other compounds. The mutants were isolated as sensitive to the antibiotic synergistin A, which is an inhibitor of protein synthesis specifically affecting a function of the 50*S* ribosome subunit (6). Although we have isolated several synergistin A-sensitive mutants, only two of these, strains H101 and H135, were studied in detail. The sensitivity of these strains to a variety of other antibiotics and compounds was determined by use of three different methods.

(i) Antibiotic disc assay. Antibiotic-impregnated discs were placed on agar plates inoculated with the appropriate bacterial strains. The plates were incubated overnight, and the zone of inhibi-

tion of growth was determined. The data obtained are summarized in Table 1. This, and the following tables list only the compounds which were selectively active at defined concentrations against one or both of the mutants but did not inhibit the parent. It should be noted that some of the antibiotics which markedly inhibited the mutants at low concentrations, and did not affect the parent at this concentration, could, however, inhibit the parent at much higher concentrations. The following compounds were also tested but they were inactive against the parent and the mutants (concentrations used in parentheses): Brij 58 (2%), lankamycin (100 μg/ml), sulfisoxazole (2 mg/disc), synergistin B (100 μg/disc), thiostrepton (100 μg/ml), and Triton X-100 (2%). The following antibiotics were equally active against the parent and mutants: Blasticidin S (50 μg/disc), bottromycin (10 μg/disc), chloramphenicol (5 μg/disc), lincomycin (500 μg/disc), pactamycin (25 μg/ml), sparsomycin (20 μg/ml), and tetracycline (30 μg/disc). It is interesting that amicetin (50 μg/disc) did not inhibit growth of H101 but 50 μg of antibiotic inhibited protein synthesis 59% (Table 2). Apparently, 50 μg per disc is not sufficient to inhibit eventual growth even though it may inhibit protein synthesis to some extent.

TABLE 1. *Sensitivity of bacterial strains to antibiotics*[a]

Antibiotic	Concn (μg/disc)	Diameter of zone of growth inhibition (mm)		
		W1895	H101	H135
Actinomycin D	100	0	*15*	0
Amicetin	50	0	0	*13*
Bacitracin	10[b]	0	*14*	0
Carbomycin	25	0	*20*	*15*
Clindamycin	20	0	*20*	*20*
Erythromycin	5	0	*11*	*11*
Fusidic acid	10	0	0	*11*
Macrocin	10	0	*8*	NT[c]
Puromycin	20	0	0	*14*
Spiramycin III	50	0	*13*	*9*
Synergistin A	25	0	*14*	*17*
Vernamycin A	25	Trace	*22*	*19*

[a] Seed agar (BBL) was inoculated with the appropriate bacterial strain and poured over solidified base agar in 150-mm plastic petri dishes. After the agar had solidified, filter discs (diameter, 7 mm) containing the indicated amounts of antibiotics were placed on the agar. The plates were incubated at 37 C overnight, and the zone of growth inhibition was determined. The results are representative of at least three separate determinations. Significant differences in antibiotic sensitivity from the parent are italicized.

[b] Ten units of bacitracin per disc.

[c] Not tested.

TABLE 2. *Ability of antibiotics to inhibit incorporation of precursors into protein or ribonucleic acid*[a]

Antibiotic	Concn (μg/ml)	Inhibition of incorporation (%)		
		W1895	H101	H135
Amicetin	50	0	59	89
Carbomycin	25	0	97	67
Erythromycin	5	6	84	NT[b]
Erythromycin	25	35	95	85
Oleandomycin	20	9	73	NT
Puromycin	10	0	2	48
Puromycin	100	27	41	99
Rifampin	10	15	98	58
Spiramycin III	25	0	97	81
Synergistin A	25	7	100	99
Vernamycin A	10	3	94	NT

[a] Cells growing in mineral salts-glucose medium containing methionine (100 μg/ml) were harvested and suspended in fresh medium containing leucine (10 μg/ml) or uracil (2.5 μg/ml), depending on the experiment. Antibiotic or water (control) was added to the cultures, and 0.5 min later 0.1 μCi of ^{14}C-leucine per ml or 0.1 μCi of ^{14}C-uracil per ml (only in the case of rifampin) was added. Samples were taken at intervals up to 30 min, mixed with an equal volume of 10% trichloroacetic acid, filtered through membrane filters (Millipore Corp.), placed in vials containing omnifluor (New England Nuclear Corp.), and counted in a model LS-100 scintillation spectrometer (Beckman Instruments, Inc.) The incorporation at 30 min in the presence of antibiotic compared to a control culture growing in the absence of antibiotic is given. The data presented are representative of at least two separate determinations.

[b] Not tested.

(ii) Inhibition of protein or RNA synthesis. The ability of a number of antibiotics to inhibit incorporation of a radioactive precursor into protein or RNA was also determined. The data obtained are tabulated in Table 2. The inhibition of incorporation at 30 min compared to a control, uninhibited culture growing in the absence of antibiotic is given.

(iii) Inhibition of growth in liquid medium. The ability of a compound to inhibit growth of the mutant and not of the wild type was noted. The minimal inhibitory concentration necessary for growth inhibition was not determined in all cases. The results are tabulated in Table 3.

Another interesting property of strain H101 is its sensitivity to detergents (Table 3). Strain H101 is (also) easily lysed by detergents during growth. In one experiment sarkosyl [Geigy Industrial Chemicals; sodium lauroyl sarcosine (0.2% final concentration)] was added to cultures of growing cells of strains W1895 and H101. As can be seen in Fig. 1, the mutant strain H101 immediately lysed, whereas the parent

TABLE 3. *Effect of antibiotics and other compounds on the growth of bacterial strains in liquid medium*[a]

Compound	Concn	Growth response		
		W1895	H101	H135
Acridine orange .	250 μg/ml	+	−	−
Deoxycholate . . .	2%	+	−	±
Erythromycin . .	2 μg/ml	+	−	NT
Oleandomycin . .	10 μg/ml	+	−	NT
Sarkosyl	0.2%	+	−	±
Sodium dodecyl sulfate	0.1%	+	−	±
Synergistin A . . .	5 μg/ml	+	−	NT

[a] Overnight cultures of the appropriate strain were diluted 1:100 by inoculation into the indicated media. Portions (5 ml) of the inoculated media were dispensed into 50-ml tubes, and selected amounts of sterile solutions of antibiotic were added. The tubes were incubated at 37 C with vigorous shaking, and growth was determined visually. The data are averages of 2 to 4 separate determinations. The data obtained for erythromycin, oleandomycin, and synergistin A were for mineral salts-glucose medium and are the minimal inhibitory concentrations. The other results were obtained by using cells growing in antibiotic medium 3 (Difco) and are not necessarily the minimal inhibitory concentrations necessary to inhibit growth. Abbreviations: +, full growth after 18 hr; ±, slight growth after 18 hr; −, no growth after 18 hr; NT, not tested.

grew normally in the medium. H135 is resistant to lysis by sarkosyl at this concentration.

Sensitivity to bacteriophage T4. *E. coli* W1895 and H135 are about equally sensitive to infection by bacteriphage T4. However, the efficiency of plating of T4 on H101 is less than 1% that on the parent, apparently because T4 does not adsorb to H101 (*data not presented*).

Colony morphology. I observed that the colonial morphology of the parent and mutants was very different (Fig. 2). The most impressive difference is that after about 24 hr growth at 37 C on mineral salts-glucose agar medium, H135 started synthesizing a mucoid substance which gives the appearance seen in Fig. 2 (arrows). This is not observed in either W1895 or H101. In many cases (but not consistently), liquid cultures of H135 made this substance at the end of the log phase of growth and became very viscous. This compound was isolated by precipitation of dialyzed (against water) supernatant fluid with cold 66% ethanol. The compound is white, insensitive to deoxyribonuclease, has no ultraviolet absorption, and reacts positively in an orcinol reaction. It probably is a polysaccharide, but it has not been further characterized.

Summary of data obtained. Table 4 is a summary of all the data obtained by one or all of the above methods. Significant differences between the mutants and the parent are italicized.

DISCUSSION

The mutants *E. coli* H101 and H135, although specifically isolated for their sensitivity to synergistin A, are also sensitive to a number of other similar (in action but not necessarily structure) and different (in action and structure) antibiotics. Strain H101 is also extremely sensitive to lysis by detergents and is resistant to bacteriophage T4. The results (summarized in Table 4) indicate that this pleiotropic mutation is probably due to a defect in the cell wall or membrane which allows compounds to enter and act on cells which are normally impermeable to these compounds. However, it is interesting that the spectrum of sensitivity is different for the two strains, although there is a great deal of overlap. H101 is sensitive to actinomycin D, bacitracin, and sarkosyl, whereas H135 is not. On the other hand, H135 is sensitive to fusidic acid, puromycin, and

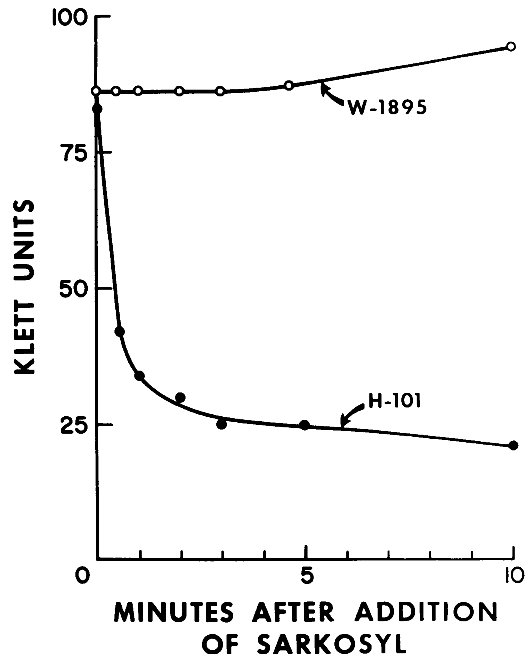

FIG. 1. *Lysis of strain H101 by sarkosyl. The cultures were grown on mineral salts-glucose medium supplemented with Casamino Acids (4). To actively growing cells, sarkosyl (0.2% final concentration) was added, and the optical density of the cultures was monitored at the indicated intervals by using a Klett-Summerson photoelectric colorimeter.* (O), *W1895;* (●), *H101. Although not shown in this figure, strain W1895 grew with a normal mass doubling time in the presence of sarkosyl.*

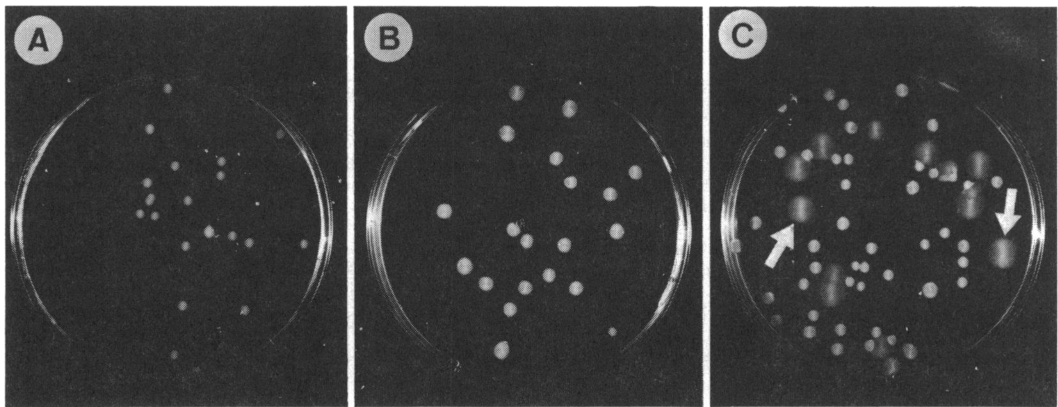

FIG. 2. *Colonial morphology of bacterial strains. The cells were plated on mineral salts-glucose agar which were incubated at 37 C for 24 hr. (A) W1895, (B) H101, (C) H135. At this time all the colonies in C do not have the same morphology. Some are mucoid (arrows) and some are not yet at this stage. Eventually all the colonies will have the mucoid appearance.*

TABLE 4. *Summary of data*[a]

Compound	Bacterial strain		
	W1895	H101	H135
Actinomycin D	R	*S*	R
Amicetin	R	*S*	*S*
Bacitracin	R	*S*	R
Carbomycin	R	*S*	*S*
Clindamycin	R	*S*	*S*
Erythromycin	R	*S*	*S*
Fusidic acid	R	R	*S*
Macrocin	R	*S*	NT
Oleandomycin	R	*S*	NT
Puromycin	R	R	*S*
Rifampin	R	*S*	*S*
Spiramycin III	R	*S*	*S*
Synergistin A	R	*S*	*S*
Vernamycin A	R	*S*	*S*
Acridine orange	R	*S*	*S*
Deoxycholate	R	*S*	+
Sarkosyl	R	*S*	R
Sodium dodecyl sulfate	R	*S*	+
Bacteriophage T4	S	*R*	S

[a] Sensitivity of strains to the various antibiotics and compounds was determined on the basis of one or more of the methods described in the previous figures and tables. Significant differences in sensitivity as compared to the parent strain W1895 are italicized Abbreviations: R, resistant, the strain is not inhibited by the appropriate concentration of drug; S, sensitive, the strain is inhibited; +, slight growth in the presence of these drugs (these strains are more sensitive to the drug than the parent but less sensitive than H101); NT, not tested.

bacteriophage T4 but H101 is not. The reason for this is not apparent.

Although these specific mutants have not been previously described, they are not unique. Other antibiotic-sensitive mutants have been isolated (2, 8, 10, 12), but they have not been analyzed for the spectrum of their antibiotic sensitivity. Another group of mutants, resistant or tolerant to colicins, show sensitivity to a number of antibiotics and detergents and seem to possess a membrane deficient in a protein or proteins found in the normal parent (5, 7, 11).

The present investigation is important for three reasons. First, I have described a simple method which may be useful in isolated cell wall or membrane mutants. Second, I now have several *E. coli* strains sensitive to a large number of antibiotics, which have obvious advantages in facilitating further work on the synthesis of macromolecules in intact cells. Third, strain H101, because it is easily lysed by detergents, can be used for the rapid isolation of enzymes, polysomes, or deoxyribonucleic acid membrane complexes (13) with a minimum of manipulation.

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LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by *N*-methyl-*N'*-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788-795.
2. Apirion, D. 1967. Three genes that affect *Escherichia coli* ribosomes. J. Mol. Biol. **30**:255-275.
3. Cohen, P. S., and H. L. Ennis. 1965. The requirement for potassium for bacteriophage T4 protein and deoxyribonucleic acid synthesis. Virology **27**:282-289.

4. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60:**17-28.
5. DeZwaig, R. N., and S. E. Luria. 1967. Genetics and physiology of colicin-tolerant mutants of *Escherichia coli*. J. Bacteriol. **94:**1112-1113.
6. Ennis, H. L. 1966. Inhibition of protein synthesis by polypeptide antibiotics. III. Ribosomal site of inhibition. Mol. Pharmacol. **2:**444-453.
7. Holland, I. B., E. J. Threlfall, E. M. Holland, V. Parby, and A. C. R. Samson. 1970. Mutants of *Escherichia coli* with altered surface properties which are refractory to colicin E2, sensitive to ultraviolet light and which can also show recombination deficiency, abortive growth of bacteriophage λ and filament formation. J. Gen. Microbiol. **62:**371-382.
8. Imae, Y. 1968. Mitomycin C-sensitive mutant of *Escherichia coli* K-12. J. Bacteriol. **95:**1191-1192.
9. Leive, L. 1968. Studies on the permeability change produced in coliform bacteria by ethylenediamine tetraacetate. J. Biol. Chem. **243:**2373-2380.
10. Nakamura, H. 1968. Genetic determination of resistance to acriflavine, phenethyl alcohol and sodium dodecyl sulfate in *Escherichia coli*. J. Bacteriol. **96:**987-996.
11. Onodera, K., B. Rolfe, and A. Bernstein. 1970. Demonstration of missing membrane proteins in deletion mutants of *E. coli* K12. Biochem. Biophys. Res. Commun. **39:**969-975.
12. Sekiguchi, M., and S. Iida. 1967. Mutants of *Escherichia coli* permeable to actinomycin. Proc. Nat. Acad. Sci. U.S.A. **58:**2315-2320.
13. Tremblay, G. Y., M. J. Daniels, and M. Schaechter. 1969. Isolation of a cell membrane-DNA-nascent complex from bacteria. J. Mol. Biol. **40:**65-76.