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Colicinogeny of *Escherichia coli* MRE 600

ERIKA SALAJ-ŠMIC
Institute "Ruder Bošković," 41001 Zagreb, Croatia, Yugoslavia

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Escherichia coli MRE 600 is a colicinogenic bacterium. Its colicinogenic activity may be ascribed mostly to colicin E1, although it produces a small amount of another, as yet unidentified, colicin.

Escherichia coli MRE 600 was selected from 13 strains that were examined for ribonuclease content. This strain displayed negligible ribonuclease activity (1). Because of its lack of ribonuclease I, it is widely used for experimental work in many laboratories. In addition, it is used for isolation of some bacterial tRNA's and enzymes for commercial purposes (Boehringer Mannheim, biochemicals catalog, 1976/1977). It is interesting that despite considerable usage of this strain, its colicinogenic property was not noted, even though it had been used in experiments with colicins (9). This report is the first demonstration of its colicinogeny.

The production of colicin(s) by *E. coli* MRE 600 bacteria was detected by the double-layer technique of Fredericq (3). *E. coli* MRE 600 colonies, developed after 48 h of incubation on nutrient agar plates (8 g of nutrient broth [Difco], 5 g of NaCl, and 12 g of agar [Difco] per liter of water), were killed by chloroform vapor, and then molten soft agar with sensitive bacteria was poured over the solid surface. (As a sensitive strain, *E. coli* C600 Sm^r, obtained from W. S. Sly and carried in our collection for many years, was used in all experiments.) After 24 h of incubation, an inhibition zone 22 mm in diameter was formed in the confluent lawn of sensitive bacteria.

When *E. coli* MRE 600 bacteria, killed by chloroform, were seeded together with the indicator strain in the soft agar and poured over a solid nutrient surface, tiny distinct clearings (lacunae) developed.

To identify colicin(s) produced by the *E. coli* MRE 600 bacterium, resistant mutants lacking receptors (receptor mutants) for MRE 600 colicin(s) were first isolated. This was done as described by Davies and Reeves (2). The next step was to find out whether the loss of receptors for MRE 600 colicin(s) was accompanied by a loss of receptors for any of the known colicins. For this purpose, sensitivity against colicins was examined by cross-streaking against bacteria that produced the following colicins: A, E1, E2, E3, K, L, S4, X, B, M, V, Ia, Ib, D, G, and H. (The bacterium producing colicin L was kindly obtained from P. Reeves; all other colicinogenic strains were kindly supplied by P. Fredericq.) It turned out that our resistant mutants, in contrast to their parental *E. coli* C600 strain, became resistant to colicins E1, E2, E3, and A, as well as to phage BF 23 (the phage culture was kindly supplied by P. Reeves). Such mutants are called Bfe mutants (7). Since only bacteria resistant to colicin A are sensitive to the BF 23 phage (10), whereas bacteria lacking receptors for colicin E are always Bfe resistant (like our resistant mutants), we infer that *E. coli* MRE 600 produces one of the E colicins.

To support this idea, mutants resistant to E colicins (Bfe mutants) were isolated and tested with respect to their sensitivity to *E. coli* MRE 600 colicin(s). All of them became partially resistant to the MRE 600 colicin(s), but full cross-resistance did not occur. Thus, the *E. coli* MRE 600 bacterium, in addition to one of the E colicins, also produces another colicin; i.e., it is a multicolicinogenic strain.

To confirm this, electrophoresis in agar gel was done according to Davies and Reeves (2). After electrophoresis had been carried out, indicator bacteria in soft agar were poured over the agar gel. After overnight incubation, an elongated inhibition zone, with a distinct trailing, appeared. When electrophoretically separated colicins were then covered with Bfe bacteria, the inhibition zone was considerably reduced; however, a small area of inhibition (corresponding to the fast-moving component) was still present. This indicates that the *E. coli* MRE 600 bacterium produces at least two colicins, one of which belongs to group E.

When the colicin preparation from a mitomycin-induced culture of *E. coli* MRE 600 was purified by fractional precipitation with ammonium sulfate (11), colicin E contributed more than 99% of the total colicinogenic activity, as shown by the spot test (3). Therefore, we further characterized the "E component" of the MRE

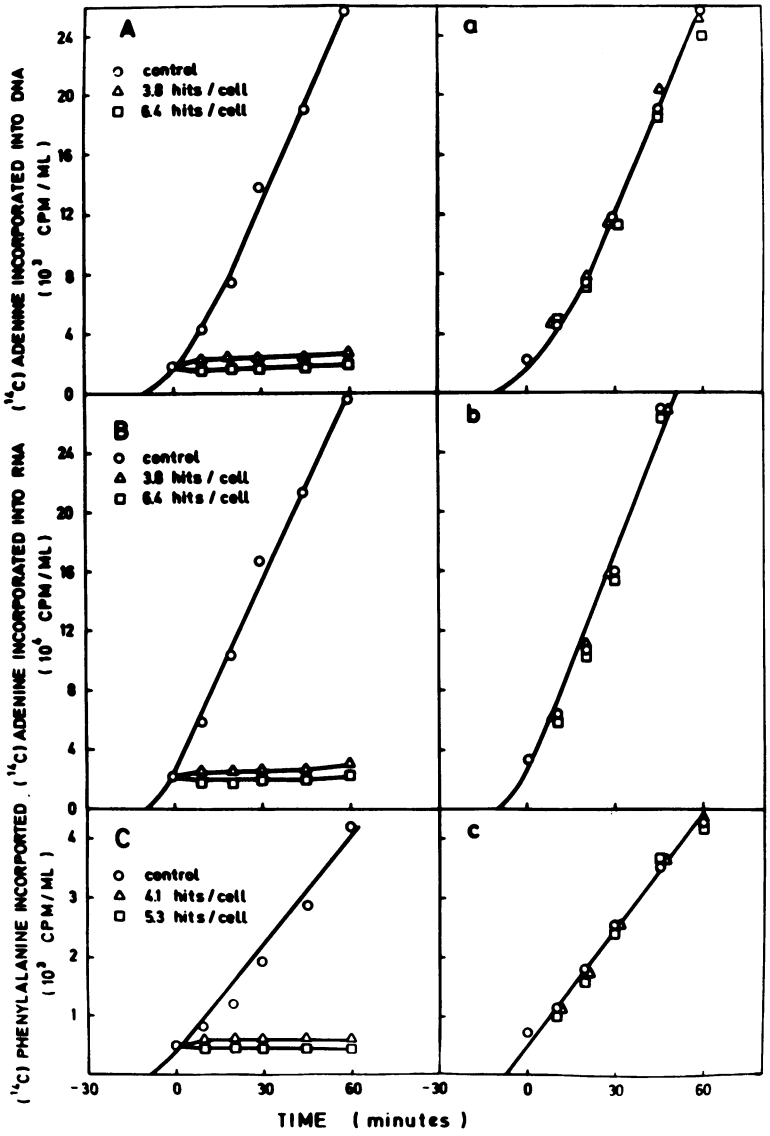

FIG. 1. *Effect of E. coli MRE 600 colicin preparation on DNA, RNA, and protein syntheses in E. coli C600 and E. coli Bfe cells. E. coli C600 (A, B, and C) and E. coli C600 resistant to E colicins (a, b, and c) were grown at 37°C in Casamino Acids-supplemented minimal medium (6) to a concentration of 10^8 cells per ml. To determine DNA (A, a) and RNA (B, b) syntheses, a portion of each bacterial culture was labeled with 0.25 μCi of [^{14}C]adenine per ml (specific activity, 0.22 mCi/mmol). The amount of radioactive precursor incorporated into DNA and RNA was determined as described by Kennedy (8). To determine protein synthesis (C, c), 0.20 μCi of [^{14}C]phenylalanine per ml (specific activity, 513 mCi/mmol) was added, and incorporation was determined as described by Timmis and Hedges (12). In each case the cells were treated with the colicin 10 min after addition of the respective radioactive precursors (zero time of the experiment). The number of hits per cell was calculated on the basis of the 15-min action of colicin. Symbols in (a), (b), and (c) refer to the same amounts of colicin as in (A), (B), and (C).*

600 colicin preparation by studying the effects of the colicin preparation on DNA, RNA, and protein syntheses and DNA breakdown.

No effect on DNA, RNA, and protein syntheses was observed after addition of the colicin preparation to the mutant, which was resistant to E colicins. In contrast, the treatment of sensitive cells leads to the cessation of DNA,

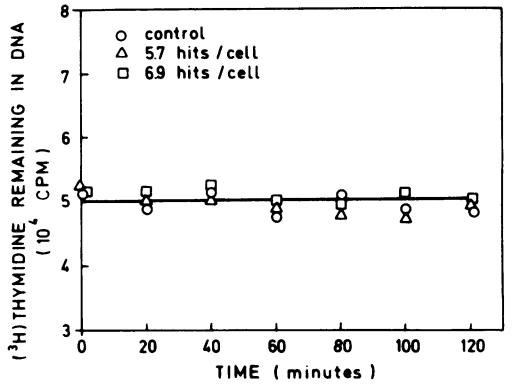

FIG. 2. *Effect of E. coli MRE 600 colicin preparation on DNA degradation in E. coli C600 cells. Bacteria were labeled in their DNA by overnight growth in Casamino Acids-minimal medium (6) supplemented with 50 μCi of [^{3}H]thymidine and 250 μg of deoxyadenosine per ml. Samples of the log-phase culture were treated with the indicated amounts of colicin at zero time of the experiment. For determination of acid-precipitable radioactivity, 0.1-ml samples were collected on Whatman 3MM filter disks soaked in 0.3 N NaOH and thereafter treated with cold 5% trichloroacetic acid, ethanol, and ether, put into scintillation vials, and counted.*

RNA, and protein syntheses (Fig. 1). The breakdown of DNA, however, was not observed (Fig. 2). Within group E, these biochemical effects are characteristic for colicin E1 (5). We may therefore conclude that the *E. coli* MRE 600 bacterium produces colicin E1 and traces of another, as yet unidentified, colicin.

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