Expression of Colicin Factor E2-P9

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Summary. In cultures of Escherichia coli K-12 or Salmonella typhimurium LT2 carrying the extra-chromosomal plasmid, colicin factor E2-P9, the incidence of cells forming colicin varied spontaneously between a minimum of ca. 0.01% in the exponential phase of growth and a maximum of ca. 75% in the stationary phase. The number of copies of the plasmid per chromosome remained constant throughout, judging from direct measurement of plasmid DNA and from transduction frequencies. The changes observed therefore reflect changes in the frequency with which each copy is expressed. Individual copies of the colicin factor in a given cell are probably expressed simultaneously, not independently. Colicin producing cells, although killed, do not disintegrate during several hours of incubation.

Introduction

Increased expression of an extra-chromosomal genetic element in bacteria offers complex problems inasmuch as it can follow from the mechanisms recognized for chromosomally-located genes; from gene dose effects, as in vegetative phage growth or in plasmid replication under relaxed control; and from concomitant processes like escape synthesis. One such genetic element is the bacterial plasmid, colicin factor E2-P9. This is a small double-stranded DNA molecule (mol. wt. 5–6 × 10^6: Bazaral and Holinski, 1968; Clowes, 1972) originally detected in a strain of Shigella sonnei, P9, which formed an E2 colicin (Fredericq, 1950; Lewis and Stocker, 1965). This plasmid has never been shown to integrate in the bacterial chromosome. Although non-transmissible alone by conjugation, it can be co-transferred by self-transmissible plasmids like colicin factor I to other enterobacteria like Escherichia coli K-12 or Salmonella typhimurium LT2 (Smith, Ozeki and Stocker, 1963). Synthesis of colicin E2 is lethal to the cell (Ozeki, Stocker and de Margerie, 1959) and is repressed under normal conditions. However, a useful feature of ColE2-P9 is that the percentage of cells synthesizing colicin at a given time can be estimated by lacuna counts, whereas no lacunae are produced by free colicin after its release from the cells (Ozeki et al., 1959). When lacuna counts are made on broth cultures of strains carrying ColE2-P9, the percentage of lacuna-forming cells fluctuates spontaneously 1000 fold, according to the stage of growth, from a minimum of ca. 0.01% to a maximum of ca. 75% (Hardy and Meynell, 1972a) and it is the causes of these gross changes in expression which are discussed here.

Methods

Culture Media. Nutrient agar was Oxoid Blood Agar Base. Nutrient broth was Oxoid Nutrient Broth No. 2. YE broth was phosphate-buffered citrate-free salts medium supplemented with tryptophan (50 μg/ml), cysteine (50 μg/ml), acid-hydrolysed casein (5 mg/ml) and yeast extract (5 mg/ml), pH 6.8.
Bacteria. Strains carrying ColE2-P9 included derivatives of:

(a) *Escherichia coli* K-12 (see Bachmann, 1972): KH293 (i.e. strain AB1157 made resistant to colicin E2-P9); M907 (i.e. strain J6-2 made resistant to colicin E2-P9, colicin K-235 and nalidixic acid, 100 µg/ml; and also carrying ColK-235); and KH371 (i.e. strain PA505 of Knüsel and Schiess, 1970) made resistant to colicin E2-P9; and

(b) *Salmonella typhimurium* LT2: KH353 (i.e. strain M827 of Spratt, Rowbury and Meynell, 1973, which lacks plasmid DNA: Lemoine and Rowbury, 1974); M910 (his-152 str-r); and M2186 (adeC-7). The recipient for transduction by phage P22 was strain M864 (rectA22 trpE2) lysogenized by a non-excluding (x, sie) mutant of phage P22 (Walsh and Meynell, 1967) to increase transduction frequencies (Ebel-Tipsis and Botstein, 1971).

Colicin indicator strains were all derived from *Escherichia coli* K-12 and included M441 (i.e. strain J6-2 resistant to nalidixic acid, 100 µg/ml); M874 (i.e. strain AB1157 lysogenized by phage λ and resistant to colicin K-235); and CL145 (i.e. strain 88-161 F- str-r and resistant to colicin E2-P9).

**Transducing Phage.** A mutant, HT104/2, of c+ phage P22 was used which had been selected by Schmieger (1971) for high-frequency transduction of chromosomal genes.

**Lacuna Counts.** Samples of culture were mixed thoroughly with a few drops of chloroform and incubated at 37 °C for 25 min. Aliquots of 1.0 ml from successive ten-fold dilutions were then added to 2.0 ml molten half-strength Oxoid Blood Agar Base held at 48 °C which contained nalidixic acid (100 µg/ml) and also two drops of an unshaken overnight broth culture of the colicin indicator strain, the mixture being poured immediately on a plate of full-strength Blood Agar Base also containing nalidixic acid, 100 µg/ml. When a mixed indicator was used, two drops of each indicator strain were added. Lacunae were counted after overnight incubation at 37 °C.

The numbers of lacunae observed in replicate counts of a given chloroformed culture differed considerably more than was attributable to chance alone since their variance was ca. three times their mean.

**Colicin Titrations by Radial Diffusion.** Colicins, like other antibacterial agents, can be assayed by measuring the size of inhibition zone produced by diffusion of colicin from a well cut in a plate of nutrient agar inoculated with sensitive indicator bacteria. However, due to the slow diffusion of colicins through agar, it is necessary to give time for the colicin to diffuse from the well before allowing the indicator to grow (Richardson, Emslie-Smith and Senior, 1968; Mayr-Harting, Hedges and Berkeley, 1972).

To prepare the plates, 15 ml nutrient agar with nalidixic acid (100 µg/ml) were inoculated with 0.15 ml of a 1/5 dilution of an unshaken overnight broth culture of the indicator, M441, and poured at once into a conventional plastic petri dish of 90 mm diameter. Four holes of 11 mm diameter were cut with a cork borer in each plate and their bases sealed to the floor of the dish with a small drop of half-strength water agar. The plates were usually left at 4 °C for 5 hr, when 80 µl of the colicin preparation was added to each well with a Hamilton microsyringe and the plates returned to 4 °C for another 20–24 hr. They were then placed at 37 °C for ca. 18 hr to allow growth of the indicator. The diameter of each inhibition zone was measured to the nearest 0.1 mm through the back of the petri dish, using vernier calipers, and was taken to be the average of two measurements at right angles.

Each sample of colicin was distributed to one well in each of four plates. Since the relation between colicin concentration and zone diameter varied from experiment to experiment, each assay also included a standard: that is, a high titre colicin preparation of which eight two-fold dilutions were each titrated in four wells in order to determine the slope of the dose-response curve for that day. Assays done on the same day were therefore comparable but not necessarily those done on different days. The logarithm of colicin concentration was found to be proportional to zone diameter (Fig. 6). Therefore the measured zone diameters were usually plotted, as in Fig. 7, and the corresponding relative concentrations of colicin calculated in arbitrary units from the response to the standard.

**Gradients.** All lysates were prepared with n-dodecylamine and sarkosyl (Meynell, 1971). Ethidium bromide gradients were performed as in Dowman and Meynell (1970) and sucrose gradients (5–20%, pH 8) as in Hardy et al. (1973).

**Definitions.** The % LFC is: 100 × lacuna count/ml divided by (lacuna count/ml + colony count/ml), since a cell cannot form both a lacuna and a colony (see Discussion). The %