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New Hemolysin (γ) Produced by *Escherichia coli*

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A new hemolysin (γ) of *Escherichia coli*, active in the absence of viable bacteria, has been recognized in mutants resistant to nalidixic acid. Nalidixic acid affects either the production or release of the hemolysin.

Two hemolysins designated α and β are recognized in *Escherichia coli*. The α-hemolysin is produced by more strains than the β-hemolysin from which it can be distinguished by its presence in cell-free filtrates (1). In some cases, a transmissible genetic element (Hly) is responsible for the production of an α-hemolysin (2).

We have recognized a new hemolysin produced by *E. coli*. When a spontaneous mutant of *E. coli* RC 709 (K_{12} F^- met^- pro^-) (3), selected for resistance to 100 μg of nalidixic acid/ml, was plated on 5% horse blood-agar, it was found to be hemolytic. The zone of hemolysis produced by this strain (709-1) did not extend beyond the borders of the colonies but was seen only when the colonies were removed from the surface of the medium. Nonhemolytic *E. coli* strains produce either no change or a slight green discoloration of the blood beneath the colony. Mutants of 709-1 which did produce zones of hemolysis beyond the colony boundary were found at a frequency of one in approximately 10^4 colonies examined. The effect on horse blood-agar of one of these double mutants (709-1-1) was compared to that of strain 709 (Fig. 1). Strains 709-1 and 709-1-1 both produced hemolysis in the absence of nalidixic acid, but the size of the zone and the intensity of the hemolysis produced by 709-1-1 was markedly enhanced by the presence of nalidixic acid in the medium. When a surface streak of 709-1-1 was removed and the remaining cells were destroyed by chloroform vapor, hemolysis was produced in a subsequent overlay of horse blood-agar (with or without nalidixic acid) only when nalidixic acid had been added to the initial growth medium. This demonstrated that (i) the hemolysin is active in the absence of viable bacteria, and (ii) nalidixic acid affects the production or release of the hemolysin and not the integrity of the erythrocytes. Whereas strains producing the α- and β-hemolysins affected erythrocytes of all species tested, strains 709-1 and -1-1, grown in the presence or absence of nalidixic acid, did not affect human or rabbit erythrocytes (Table 1). These findings indicate that strains 709-1 and -1-1 produce a previously unrecognized hemolysin, which we suggest be designated γ.

Spontaneous nalidixic acid-resistant mutants of other nonpathogenic and pathogenic strains of *E. coli* were examined, and all were found to hemolyze horse erythrocytes as does strain 709-1. In contrast, none of the spontaneous nalidixic acid-resistant mutants obtained from strains of *Salmonella* and *Shigella* had any hemolytic activity against horse erythrocytes. The relationship between the genetic determinant for nalidixic acid resistance and γ-hemolysin production in *E. coli* and the phenotypic effect of nalidixic acid on the hemolytic activity of strain 709-1-1 have not yet been clearly defined. The nutritional requirements of strains 709-1 and -1-1 and *E. coli* AB 1932-1 (K_{12} F^- arg^- met^- xyl^- gal^- lac^- Nal^R), another spontaneous nalidixic acid-resistant mutant which produces γ-hemolysin, were identical to those of their parent strains,

TABLE 1. *Hemolytic activity of strains producing α, β, and γ hemolysis against erythrocytes of different species*

Type of erythrocyte	Hemolytic activity[a]		
	α	β	γ
Guinea pig	+	+	+
Horse	+	+	+
Human	+	+	−
Rabbit	+	+	−
Sheep	+	+	+

[a] Bacteria were streaked on tryptic digest-agar containing 5% erythrocytes; plates were examined for hemolysis after 24 hr of incubation at 37 C. Hemolysis present, +.

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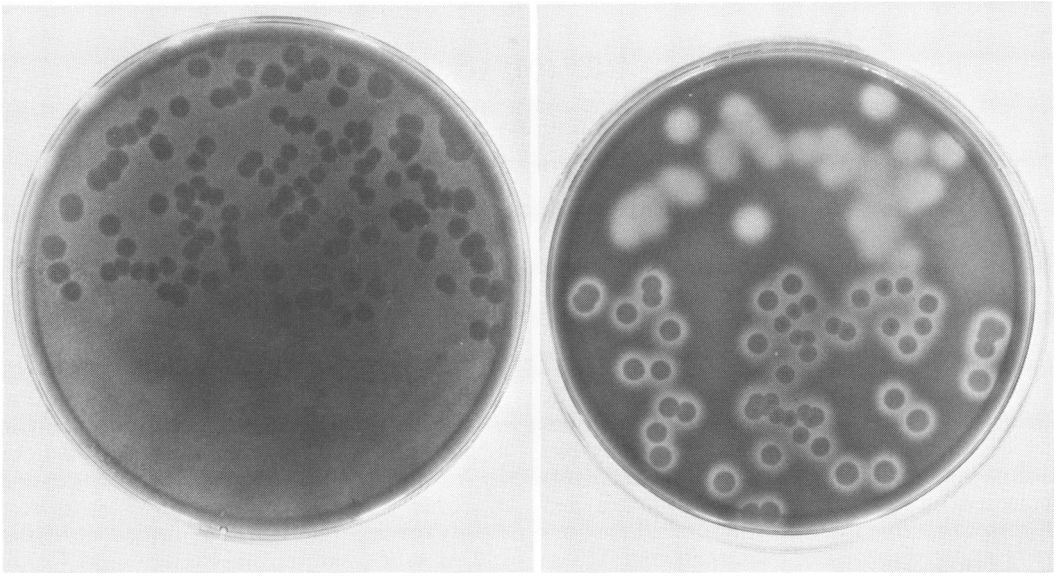

FIG. 1. *Comparison of the hemolytic effect, on horse blood-agar, of the parent strain 709 (left) with the mutant strain 709-1-1 (right). The bacterial growth was removed from half the plate to contrast more clearly the hemolytic effect.*

whether grown in the presence or absence of nalidixic acid. These findings indicate that the production of γ-hemolysin results from some specific effect of the mutation to nalidixic acid resistance and not from an indirect association with the nonspecific mutagenic action of nalidixic acid (4). A more detailed analysis of the genetic regulation of the γ-hemolysin will be presented elsewhere.

These findings may have practical as well as theoretical importance, since hemolysin production has been associated with those strains of *E. coli* most commonly found to produce extra-intestinal disease in man (K. L. Vosti, *personal communication*); moreover, they should be taken into account by diagnositc laboratories which use hemolysis to help differentiate *E. coli* strains.

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