INFECTION AND IMMUNITY, May 1979, p. 562-564
0019-9567/79/05-0562/03$02.00/0

Vol. 24, No. 2

NOTES

Relationship Between Colicin V Activity and Virulence in *Escherichia coli*

ROBERT L. QUACKENBUSH†* AND STANLEY FALKOW

Department of Microbiology and Immunology-SC42, University of Washington School of Medicine, Seattle, Washington 98195

Received for publication 5 February 1979

Colicin V activity is not essential to ColV plasmid-mediated virulence enhancement in *Escherichia coli*.

Colicin V is genetically determined by various types of plasmids (ColV plasmids) that occur in strains of *Escherichia coli*. ColV plasmids vary in their molecular mass and ability to act as transfer factors (1, 2, 7, 12; personal observations). Smith and Huggins (13) observed that there is significant association in *E. coli* between the possession of a ColV plasmid and the ability to cause septicemia, particularly in domestic animals such as cattle and chickens. Smith (12) further observed that the virulence of several strains of *E. coli*, with respect to their ability to cause sepsis in laboratory animals, was increased when ColV plasmids were introduced and decreased when ColV plasmids were cured. These observations have been confirmed by Ozanne et al. (10), who have also reported that a small, dialyzable protein, identified as colicin V, exhibited inhibitory activity against macrophages (11).

The possibility that colicin V may act to enhance the virulence of strains of *E. coli* with respect to their ability to cause sepsis in certain warm-blooded animals was strongly indicated. The purpose of the present investigation was to determine whether colicin V activity was necessary for the enhancement of virulence by strains of *E. coli* that carry ColV plasmids. The basic approach is to compare the virulence of colicin V-producing *E. coli* with isogenic strains that carry ColV plasmids in which the genes for colicin V synthesis have been inactivated. The colicin V-negative mutants were obtained by selecting for transposon-mediated mutations (3-6).

Bacterial strains used were *E. coli* UB1636 and *E. coli* B188ColV and its derivatives (Table 1). *E. coli* B188ColV carries a non-self-transferable ColV plasmid that is known to contain genes encoding for virulence enhancement (13). *E. coli* UB1636 (isolated and kindly provided by Martin Robinson, Sandoz Forschungsinstitut, Vienna, Austria) carries the plasmid pMR5, which is a derivative of RP4 with a temperature-sensitive mutation in a replication gene. Like RP4, pMR5 contains the transposition element Tn*1*. When strains of *E. coli* that carry pMR5 are grown at 42°C in the presence of ampicillin or carbenicillin, replication of pMR5 is inhibited, thus favoring the growth of cells in which Tn*1* has transposed into other deoxyribonucleic acid molecules. Transposition of Tn*1* from pMR5 into other plasmids has been observed to occur approximately 1,000 times more frequently than do insertions of Tn*1* into the chromosome (M. Robinson, personal communication).

Strains of *E. coli* containing Tn*1* inserted into ColV plasmid deoxyribonucleic acid were constructed as follows. Transfer of pMR5 into a ColV plasmid-containing strain of *E. coli* was achieved by mating *E. coli* UB1636 (pMR5) with B188ColV on a Trypticase soy agar (BBL Microbiology Systems) plate overnight at 32°C. Transconjugants were selected by inoculating brain heart infusion (Difco Laboratories) containing nalidixic acid (30 μg/ml), kanamycin (30 μg/ml), and ampicillin (30 μg/ml) with bacteria scraped from the mating plate and incubating for 24 h at 32°C, followed by transfer to MacConkey agar plates containing the same concentrations of antibiotics. A mid-log-phase broth culture of the transconjugant strain *E. coli* SF1000 (ColV, pMR5) was then incubated at 42°C to select cells in which transpositions of Tn*1* from pMR5 into the ColV plasmid occurred. After 2 h, dilutions of the broth culture were spread onto MacConkey agar plates containing

† Present address: Department of Microbiology, School of Medicine, University of South Dakota, Vermillion, SD 57069.

nalidixic acid (30 μg/ml) and carbenicillin (250 μg/ml) and incubated overnight at 42°C.

Two hundred clones were screened for sensitivity to kanamycin and the ability to produce colicin V. Colicin V production was detected by overlaying Trypticase soy agar plates that were spot inoculated with clones derived from *E. coli* SF1000 with Trypticase soy agar containing *E. coli* C600 and observing which clones inhibited the growth of *E. coli* C600 in the overlay. The spot-inoculated plates were incubated at 37°C overnight and treated with chloroform before applying the Trypticase soy agar overlay containing *E. coli* C600. Sixty-three percent of the clones were sensitive to kanamycin and resistant to carbenicillin, indicating the loss of pMR5 and retention of Tn*1*. Approximately 12% of the clones presumed to carry Tn*1* insertions did not produce detectable levels of ColV activity.

Five of the putative colicin V-negative, ColV::Tn*1*-containing strains derived from *E. coli* SF1000 were screened for the presence of plasmid deoxyribonucleic acid as described by Meyers et al. (8). All five clones still carry plasmids of similar molecular mass (approximately 50 × 10^6 daltons) to the ColV plasmid, indicating that inactivation of colicin V was caused by mutations mediated by Tn*1* insertion events. Restriction enzyme analyses (manuscript in preparation) of plasmid deoxyribonucleic acid from these clones and from the parental strain (*E. coli* B188ColV) demonstrated that Tn*1* was inserted into the ColV plasmid and that at least three of the five plasmids analyzed have slightly different Tn*1* insertion sites. The five strains determined to be colicin V negative due to Tn*1*-mediated insertional inactivation have been designated *E. coli* SF1001 through SF1005, and their ColV::Tn*1* plasmids have been designated pBQ1 through pBQ5, respectively (Table 1).

The virulence of the colicin V-negative mutants and related strains was tested by observing the number of Swiss-Webster adult mice killed by injections of 0.1-ml portions of various cell concentrations administered intraperitoneally (Table 2). The data clearly show that those strains carrying the ColV plasmid but lacking colicin V activity (*E. coli* SF1001 through SF1005) are still more virulent than *E. coli* B188, which lacks the ColV plasmid. These colicin V-negative derivatives also produced mortality rates in mice that did not differ significantly from mortality rates produced by the parental strains *E. coli* B188ColV and *E. coli* SF1000 or by the colicin V-positive derivative strains of *E.*

TABLE 2. *Lethality of E. coli injected into adult Swiss-Webster mice*

Strain	Colicin V production	Viable cells inoculated intraperitoneally	Mortality at 18 h after injection	Mortality after 18 h (%)
B188	−	6×10^7	1/7	14.5
B188ColV	+	6×10^7	6/6	100
SF1000	+	6×10^7	6/6	100
SF1001	−	6×10^7	6/6	100
B188	−	2×10^7	0/7	0
B188ColV	−	2×10^7	7/8	87.5
SF1000	+	2×10^7	6/8	75
SF1001	−	2×10^7	7/8	87.5
SF1002	−	2×10^7	8/8	100
SF1003	−	2×10^7	8/8	100
SF1004	−	2×10^7	7/8	75
SF1005	−	2×10^7	7/8	87.5
B188ColV(Tn*1*)	+	2×10^7	26/32	81

TABLE 1. *Strains of E. coli*

Strains	Derived from:	Relevant traits	Plasmid[a]	Transposition element	Plasmid properties[a]	Sources
UB1636		*lac*⁻, Sm^r	pMR5	Tn*1*	Rep(ts), Ap^r, Km^r Tc^r, Tra$^+$	M. Robinson
B188ColV	Natural isolate	Nal^r	ColV		Cva$^+$, Tra$^-$	H. W. Smith
B188	B188ColV	Nal^r				H. W. Smith
SF1000	B188ColV	Nal^r	ColV		Cva$^+$, Tra$^-$	This study
			pMR5	Tn*1*	Rep(ts), Ap^r, Km^r Tc^r, Tra$^+$	
SF1001	SF1000	Nal^r	pBQ1	Tn*1*	Cva$^-$, Ap^r, Tra$^-$	This study
SF1002	SF1000	Nal^r	pBQ2	Tn*1*	Cva$^-$, Ap^r, Tra$^-$	This study
SF1003	SF1000	Nal^r	pBQ3	Tn*1*	Cva$^-$, Ap^r, Tra$^-$	This study
SF1004	SF1000	Nal^r	pBQ4	Tn*1*	Cva$^-$, Ap^r, Tra$^-$	This study
SF1005	SF1000	Nal^r	pBQ5	Tn*1*	Cva$^-$, Ap^r, Tra$^-$	This study
B188ColV(Tn*1*)[b]	SF1000	Nal^r	ColV(Tn*1*)[b]	Tn*1*	Cva$^+$, Ap^r, Tra$^-$	This study

[a] Plasmids and their phenotypic traits were named following the recommendations of Novick et al. (9).

[b] B188ColV(Tn*1*) is not a single strain, but four randomly selected clones, derived from *E. coli* SF1000, that produce colicin V and are presumed to carry Tn*1* insertions within their ColV plasmids.

coli SF1000 that possess Tn*1* insertions into the ColV plasmid which have no observable effect on colicin synthesis. The minimum number of cells required to kill mice (5×10^5 cells) is the same for *E. coli* B188ColV and *E. coli* SF1002, indicating that loss of colicin activity does not necessarily result in diminished virulence.

These data clearly indicate that colicin V activity itself is not essential for virulence enhancement of *E. coli*. However, since the virulence enhancement trait is associated with ColV plasmids of different molecular masses and transfer characteristics, it is still probable that the genetic determinants for virulence enhancement are closely linked to colicin V-related genes.

This work was supported by Public Health Service grant AI-1088 from the National Institute of Allergy and Infectious Diseases. R.L.Q. is supported by Public Health Service fellowship 1 P32 AIO5645-01 from the National Institutes of Health.

We thank H. William S. Smith and Ron Gill for helpful discussions.

LITERATURE CITED

1. **Clowes, R. C.** 1972. Molecular structure of bacterial plasmids. Bacteriol. Rev. **36**:361-405.
2. **Fredericq, P.** 1956. Reserches sur la frequence des souches tranductrices des propietes colicinogenes. Ct. Rd. Soc. Biol. **150**:1036-1039.
3. **Heffron, F., C. Rubens, and S. Falkow.** 1975. Translocation of a plasmid DNA sequence which mediates ampicillin resistance: molecular nature and specificity of insertion. Proc. Natl. Acad. Sci. U.S.A. **72**:3623-3627.
4. **Kleckner, N., R. K. Chan, B. K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97**:561-575.
5. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements: new methods in bacterial genetics. J. Mol. Biol. **116**:125-159.
6. **Kopecko, D. J., and S. N. Cohen.** 1975. Site-specific rec-A-independent recombination between bacterial plasmids: involvement of palindromes at the recombination loci. Proc. Natl. Acad. Sci. U.S.A. **72**:1373-1377.
7. **MacFarren, A. C., and R. C. Clowes.** 1967. A comparative study of two F-like colicin factors, ColV2 and ColV3, in *Escherichia coli* K-12. J. Bacteriol. **94**:365-377.
8. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1519-1537.
9. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168-189.
10. **Ozanne, G., L. G. Mathieu, and J. P. Baril.** 1977. Production of colicin V in vitro and in vivo and observations on its effects in experimental animals. Infect. Immun. **17**:497-503.
11. **Ozanne, G., L. G. Mathieu, and J. P. Baril.** 1977. Production de colicines V et V_2 in vitro et in vivo etude de leur action inhibitrice sur la phagocytose par des macrophages peritoneaux. Rev. Can. Biol. **36**:307-316.
12. **Smith, H. W.** 1974. A search for transmissible pathogenic characters in invasive strains of *Escherichia coli:* the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicine V. J. Gen. Microbiol. **83**:95-111.
13. **Smith, H. W., and M. B. Huggins.** 1976. Further observations on the association of the colicine V plasmid of *Escherichia coli* with pathogenicity and with survival in the alimentary tract. J. Gen. Microbiol. **92**:335-350.