ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2004, p. 3119–3121 Vol. 48, No. 8
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.8.3119–3121.2004

Inhibitory Activities of Colicins against *Escherichia coli* Strains Responsible for Postweaning Diarrhea and Edema Disease in Swine

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Received 24 February 2004/Returned for modification 13 April 2004/Accepted 26 April 2004

The efficacies of colicins E1 and N against *Escherichia coli* strains responsible for postweaning diarrhea and edema disease, two of the most prevalent disease problems for pigs in the United States, were determined in vitro. These proteins may provide an environmentally sound means for the prevention of these infections in swine.

Postweaning diarrhea and edema disease, caused by *Escherichia coli* infections, are two of the most prevalent disease problems for pigs in the United States (12). More than 43% of the large swine facilities in the United States reported *E. coli* infections among weaned pigs in 2000, and in an attempt to prevent the spread of these infections, more than 78% of these facilities reported using prophylactic antibiotic treatments (12). The strains considered primarily responsible for these infections, F4 (K88) and F18, are not well controlled by traditional prophylactic antibiotic treatments due to the frequency and spectrum of antibiotic resistance seen in these strains (2, 8) and therefore still cause substantial losses to producers, due to both mortality and morbidity. With worldwide concern over the use of prophylactic antibiotics in animal agriculture and their contribution to the spread of antibiotic resistance (3, 4, 13), the development of alternatives to conventional antibiotics is urgently needed to protect swine from these *E. coli* infections.

Colicins, a class of bacteriocins produced by, and effective against, *E. coli* and closely related species (5), hold particular promise as alternatives to conventional antibiotics used for the control of postweaning diarrhea and edema disease in pigs. Studies examining the antibacterial activity of different colicins have typically yielded qualitative rather than quantitative determination of activity by using direct spotting or overlay techniques to determine efficacy (6, 10, 11). However, in this study, we quantitatively compared the efficacy of two purified colicins (colicins E1 [ColE1] and N [ColN]) in inhibiting the growth of the *E. coli* strains responsible for postweaning diarrhea and edema disease.

Purified colicins were obtained by inoculating colicin-producing *E. coli* strains (NC50132 and NC50145) obtained from the National Collection of Type Cultures (Public Health Laboratory Service, London, England) into Luria Broth (LB) to a starting optical density at 600 nm (OD_{600}) of about 0.1 and incubating the cultures with shaking at 37°C. Colicin production was induced when cultures reached an OD_{600} of 0.9 by the addition of 0.2 U of mitomycin C (Sigma)/ml of culture. The cell-free supernatant was obtained by centrifugation 5.5 h later and concentrated by ultrafiltration across a regenerated cellulose membrane in a stir cell apparatus (Amicon, Millipore, Bedford, Mass.). The concentrated sample was then desalted against 10 mM Tris-Cl, pH 8, and purified by ion exchange chromatography using Q Sepharose (Amersham Biosciences, Piscataway, N.J.) equilibrated with 10 mM Tris-Cl, pH 8. The bound protein was eluted with a continuous NaCl gradient by using an AKTAprime chromatography system (Amersham Bioscience), and fractions containing the highest concentrations of colicin were pooled and concentrated by ultrafiltration. The protein concentrations of these pooled samples were determined (9), and the percentage of colicin was determined by

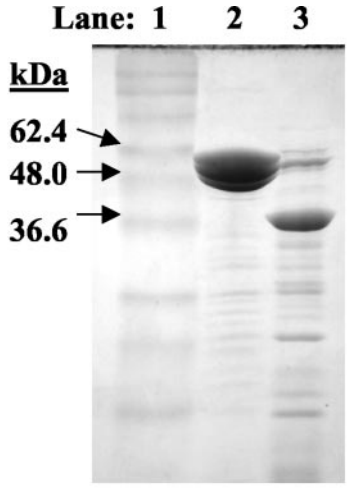

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified ColE1 and ColN. Lane 1, BenchMark prestained protein ladder (Invitrogen, Carlsbad, Calif.); lane 2, 5 μg of the purified ColE1 sample; lane 3, 14 μg of the purified ColN sample.

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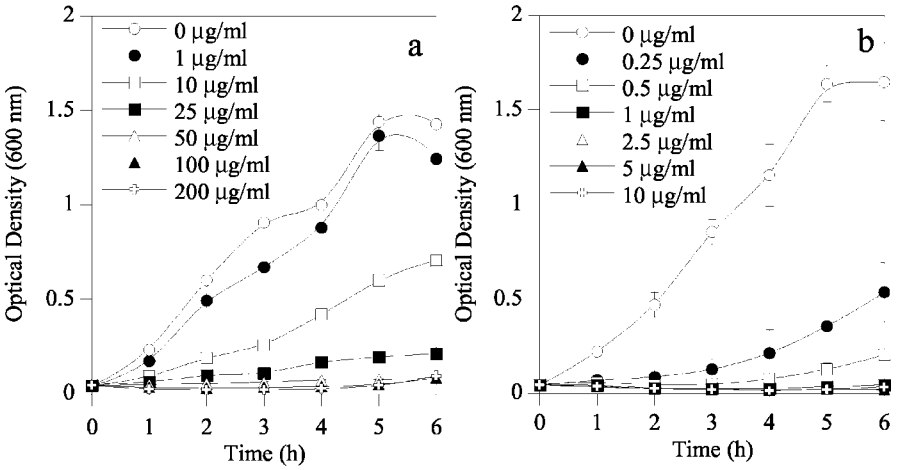

FIG. 2. Effect of ColE1 on the growth of *E. coli* F4 (K88) (a) and F18 (b).

densitometry after sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie blue staining with a 16-bit megapixel charge-coupled device camera, FluorChem 8800, and FluorChem IS800 software (Alpha Innotech, San Leandro, Calif.). Yields of 1.1 mg of purified ColN/liter of culture and 7.6 mg of purified ColE1/liter of culture were obtained. The purity of the ColN and ColE1 isolates were 30 and 85%, respectively (Fig. 1).

Quantitative determinations of the efficacies of these purified colicins against *E. coli* F4 (K88) and F18 were made by using pure cultures obtained from the culture collection at the USDA Agricultural Research Service Federal Food Safety Research Unit (College Station, Tex.). These cultures were grown overnight in LB at 37°C with shaking and then used to inoculate a flask of LB to an OD_{600} of about 0.05. The freshly inoculated LB was then aliquoted (5 ml) into culture tubes containing various colicin concentrations (Fig. 2 and 3). The volume of the colicin addition was made constant (175 μl) with 10 mM Tris, pH 8. These tubes were then incubated with shaking at 37°C, and their OD_{600} was determined hourly for 6 h. The CFU/milliliter of these *E. coli* strains were also determined by serial dilutions and direct plating on LB, both initially and 3 h postinoculation. These experiments were repeated in triplicate, and the values presented are means. ColN was more effective ($P < 0.05$) than ColE1 against *E. coli* F4 (K88). Tenfold more ColE1 than ColN was required to inhibit the growth of *E. coli* F4 (K88) (Fig. 2 and 3). After 3 h of growth in LB containing 50 μg of colicin/ml, ColN caused a 2-log reduction in CFU/milliliter, whereas ColE1 caused only a 1-log reduction (Table 1). ColE1, however, was far more effective ($P < 0.05$) than ColN against *E. coli* F18 (Fig. 2 and 3). No increase in the OD_{600} was seen during the 6-h incubation with 1 μg of ColE1/ml, whereas ColN concentrations greater than 25 μg/ml were needed to see this effect. Both 1 μg of ColE1 and 50 μg of ColN/ml caused a 1-log reduction in the CFU of *E. coli* F18/milliliter (Table 1).

The prophylactic use of antibiotics in animal agriculture has been greatly scrutinized in recent years due to concerns regarding its role in contributing to antibiotic resistance. This scrutiny has led to increased regulation over the use of antibiotics in animal agriculture (3, 4, 13) and will likely continue towards zero tolerance for the use of prophylactic or growth-

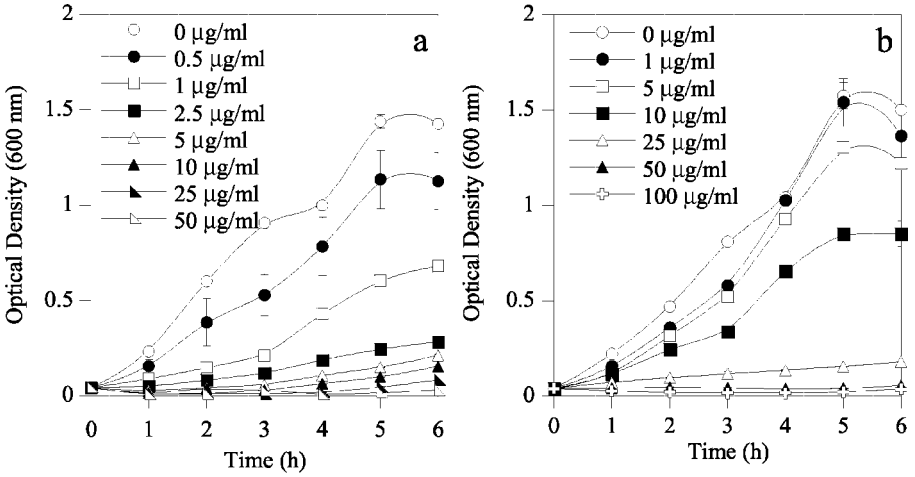

FIG. 3. Effect of ColN on the growth of *E. coli* F4 (K88) (a) and F18 (b).

TABLE 1. Effect of colicins on the viability of *E. coli* F4 (K88) and F18 after 3 h of incubation[a]

E. coli strain	ColE1		ColN	
	Dose (μg/ml)	CFU/ml	Dose (μg/ml)	CFU/ml
F4 (K88)	0	3×10^9	0	3×10^9
	50	5×10^6	10	1.1×10^7
	200	4×10^6	50	6×10^5
F18	0	2×10^9	0	2×10^9
	1	1.2×10^6	50	1×10^6
	100	5×10^4	100	4×10^5

[a] Initial CFU/milliliter were 6×10^7 and 1×10^7 for *E. coli* F4 (K88) and F18, respectively.

promoting antibiotic use in animals. Therefore, it is essential for the sustainability of animal agriculture to examine alternatives to conventional antibiotics to improve animal health and production efficiency. Because of their in vitro efficacy against *E. coli* F4 (K88) and F18, the strains responsible for causing postweaning diarrhea and edema disease in pigs, ColE1 and ColN should be examined as alternatives to conventional antibiotics in swine production. Since the modes of cell recognition and transport through the periplasm are dramatically different between ColE1 and ColN (1, 7), a combination of these colicins may be more effective than either colicin alone for their use in animal agriculture.

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