Colicin U, a Novel Colicin Produced by Shigella boydii

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A novel colicin, designated colicin U, was found in two Shigella boydii strains of serovars 1 and 8. Colicin U was active against bacterial strains of the genera Escherichia and Shigella. Plasmid pColU (7.3 kb) of the colicogenic strain S. boydii M592 (serovar 8) was sequenced, and three colicin genes were identified. The colicin U activity gene, cuu, encodes a protein of 619 amino acids (Mr, 66,289); the immunity gene, cui, encodes a protein of 174 amino acids (Mr, 20,688); and the lytic protein gene, cul, encodes a polypeptide of 45 amino acids (Mr, 4,672). Colicin U displays sequence similarities to various colicins. The N-terminal sequence of 130 amino acids has 54% identity to the N-terminal sequence of bacteriocin 28b produced by Serratia marcescens. Furthermore, the N-terminal 36 amino acids have striking sequence identity (83%) to colicin A. Although the C-terminal pore-forming sequence of colicin U shows the highest degree of identity (73%) to the pore-forming C-terminal sequence of colicin B, the immunity protein, which interacts with the same region, displays a higher degree of sequence similarity to the immunity protein of colicin A (45%) than to the immunity protein of colicin B (30.5%). Immunity specificity is probably conferred by a short sequence from residues 571 to residue 599 of colicin U; this sequence is not similar to that of colicin B. We showed that binding of colicin U to sensitive cells is mediated by the OmpA protein, the OmpF porin, and core lipopolysaccharide. Uptake of colicin U was dependent on the TolA, -B, -Q, and -R proteins. pColU is homologous to plasmid pSB41 (4.1 kb) except for the colicin genes on pColU. pSB41 and pColU coexist in S. boydii strains and can be cotransformed into Escherichia coli, and both plasmids are homologous to pColE1.

Colicins are antibacterial proteins whose genes are usually located on plasmids. The bacteriocin 28b gene of Serratia marcescens is the only colicin known to be localized on the chromosome (59). Colicins are produced by certain bacterial strains of the family Enterobacteriaceae, particularly by Escherichia coli. The toxic effects of colicins are limited to sensitive strains of this family and are most active within the species of the producer strain (43, 44). Colicins consist of single polypeptide chains with molecular masses of 29 to 75 kDa (11). Their interaction with susceptible bacterial cells occurs in three steps: attachment to a specific receptor of the outer membrane, translocation through the cell envelope, and lethal action on the cell target (2, 11, 15, 46).

The three-step mechanism of colicin action is reflected by the three-domain structure of the polypeptide. The central domain mediates binding to cell surface receptors, the N-terminal sequence is responsible for the uptake of colicins across the cell envelope, and the C-terminal part exerts the lethal effects. The domains function largely independently of each other. Nature assembled colicins by exchanging DNA segments that encode domains (39, 40, 47).

Colicins are classified according to receptor specificity, immunity, and type of translocation through the cell envelope of susceptible cells (17, 18). Group A colicins utilize the Tol system, which consists of the proteins TolA, TolB, TolQ, and TolR (55), and group B colicins use the Ton system, which consists of the proteins TonB, ExbB, and ExbD (9).

In 1992, V. Horák (31) demonstrated the production of a colicin in two cross-immune strains of Shigella boydii (serovars 1 and 8). The absence of cross-resistance with any of the known colicins suggested that this was a new type of colicin, for which, in accordance with the original Fredericq classification system (24), the name colicin U was proposed (31). This paper presents the molecular characterization of this novel colicin and its plasmid.

MATERIALS AND METHODS

Bacterial strains. E. coli strains, plasmids, and the bacteriocinophages used in the experiments are listed in Table 1. S. boydii M592 (serovar 8) and S. boydii 215/92 (serovar 1) were from V. Horák, Department of Microbiology, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic. S. boydii Shp13/56 (serovar 8) and S. boydii Shp166/58 (serovar 1) were from the Czech National Collection of Type Cultures, Prague, Czech Republic. Colicins A, B, K, N, and L were produced by E. coli BZB2101(pColA-CAS1), BZB2102(pColB-K260), BZZ2116(pColK-K235), BZB2123(pColN-284), and Serratia marcescens JF246 (J. Foulds), respectively. The BZB strains were obtained from A. P. Pugsley, Institut Pasteur, Paris, France.

Growth media. Bacteria were grown at 37°C in TY medium containing 8 g of Bacto Tryptone (Difco Laboratories), 5 g of yeast extract, and 5 g of NaCl per liter (pH 7). For maintenance of plasmids, 50 μg of chloramphenicol per ml was added to liquid medium and to 1.5% (wt/vol) TY agar.

Microbiological methods. Serial dilutions of a stock solution of bacteriophage C21 were mixed with 0.1 ml of bacteria (109 per ml) and plated to determine the number of plaques. Crude extracts of colicins were prepared from colicogenic strains grown in TY medium supplemented with mitomycin (0.5 μg per ml). Cells were harvested, suspended in distilled water, washed, and sonicated.

Colicin activity assays were performed as described previously (39). The colicin binding activities of both parental and mutant strains of E. coli were assayed by mixing 1 ml of cell suspension in distilled water (2 × 108 per ml) with 1 ml of colicin solution (103 dilution titer). The mixtures were incubated for 20 min at 37°C and then centrifuged. The supernatant was decanted, and unbound colicin was assayed by the punch-hole method (36, 58).

Recombinant DNA methods. Standard techniques were employed for restriction endonuclease analysis, ligation, isolation of plasmids, and transformation of plasmid DNA (48). pColU genes were cloned into the vector pBCKSK+ (Stratagene). DNA was sequenced by using the dye-coupled chain termination method (49), fluorescence-labeled nucleotides (AutoRead Sequencing Kit), and the A.L.F. sequencer. Site-specific mutagenesis was performed by PCR (35). The mutated fragments were examined by DNA sequencing.
Protein analytical procedures. Proteins were labeled radioactively in *E. coli* BL21 (54) with [35S]methionine (56) and were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (40). Proteins were labeled in vitro with [35S]methionine in a bacterial cell-free transcription-translation system (Promega, Madison, Wis.).

SDS-PAGE of LPS. Lipopolysaccharide (LPS) samples were prepared by using the SDS–proteinase K–whole-cell lysate method (29). Samples were run on 17% polyacrylamide gels, and then the gels were silver stained (57).

Computer-assisted sequence analysis. Computer-assisted sequence analysis was performed as described previously (39).

Nucleotide sequence accession number. The nucleotide sequences reported in this study were deposited in the EMBL data bank under accession no. Y11823.

### RESULTS

**Genes on the pColU plasmid.** *E. coli* 5K was transformed with the colicin U-determining plasmid of *S. boydii* M592 (serovar 8). Selection for immunity to added colicin U resulted in the isolation of transformants that carried two plasmids: a 7.3-kb plasmid designated pColU and a 4.1-kb plasmid designated pSB41. No transformants with a single plasmid were obtained. One such transformant was chosen for further studies and was designated strain UST19. Sequencing of one DNA
strand of each plasmid revealed partial sequence identity and, in addition, similarity to the pColE1 sequence. Sequence similarity between pSB41 and pColE1 was high in the rep and mob regions of pColE1 (bp 596 to 3733) (Fig. 1) (14). The sequence of the remaining 1-kb region of pSB41 was not similar to sequences of pColE1, with the exception of a 0.1-kb sequence, which was similar to that of exc from pColE1. pColU and pColE1 had high sequence similarity from bp 496 to 5140, extending from the second third of the gene for the lytic protein (see below) to the start codon of the colicin activity gene (Fig. 1). The remaining 2.6-kb pColU region did not show any sequence similarity to pColE1. The pSB41 and pColU sequences differed in the origin of replication, which may allow their coexistence in cells.

Transformation of pColU alone was achieved after the plasmid was cleaved by ClaI and ligated into pBCSK+, which resulted in pDS1. The cloned 2.3-kb HindIII-EcoRV fragment on pDS3 (Table 1) encoded the gene necessary for colicin U synthesis. However, the transformant carrying this fragment was not stable because, as sequencing revealed, it lacked the immunity gene. The SpeI-ClaI (2.7 kb) fragment in pDS4 conferred immunity to colicin U, which indicated the presence of a functional immunity gene on the cloned fragment. Bacteria carrying the cloned EcoRV-EcoRV fragment (1.5 kb) in pDS5 either grew or lysed, depending on the polarity of the colicin U lytic gene relative to the lacZ promoter of pBCSK+.

pColU was sequenced between HindII and AluI (2.9 kb) on both strands. Three open reading frames, one each for colicin U activity (cua), immunity (cui), and lysis (cul), were identified (Fig. 2). The cui transcription polarity is opposite to that of cua and cul, which is characteristic for colicins whose target is the cytoplasmic membrane. The cua gene codes for a protein consisting of 619 amino acid residues (Mr, 66,289), cui codes for a protein of 174 residues (Mr, 20,688), and cul codes for a polypeptide of 45 residues (Mr, 4,673).

The promoter region upstream of cua contains two overlapping SOS boxes, as is found on pColE1, and a highly conserved -35 region and a less conserved -10 region, as is observed in other colicin determinants. The promoter region from pDS1 was cloned into pDS2 by using HindII, which replaced the optimal -35 consensus region TTAGCA with TCGACA and resulted in a considerable decrease of colicin U synthesis upon SOS induction (data not shown).

SDS-PAGE of radiolabeled proteins from cells synthesizing the colicin activity and immunity proteins showed bands that corresponded to the expected size of the proteins (Fig. 3, lane 1 [65 kDa], and lane 3 [21 kDa]).

pColU of another S. boydii strain was selected for compar-
FIG. 2. Nucleotide sequence of the *Hind*II-*Alu* fragment (2.9 kb) of pColU. The arrows indicate the transcriptional polarities of the *cua*, *cui*, and *cul* genes. Putative promoter regions (−10 and −35), SOS boxes, ribosome binding site sequences (*S.D.*), transcription termination sites (T1, T2, T3), and a predicted TolA box are indicated.
The restriction maps of the pColU plasmids and the nucleotide sequences of the cua, cui, and cul genes were identical.

Colicin U protein. A comparison of the colicin U amino acid sequence with those of known colicins showed the highest degree of similarity to colicin A (36.8% identity, 10.1% similarity). However, the degree of sequence similarity varied along the polypeptide chain. A dendrogram of sequence similarities of pore-forming colicins is shown in Fig. 4. Since colicins are composed of domains, the degree of sequence similarity was determined for each domain. The 200 C-terminal amino acids of colicin U exhibited the greatest sequence similarity to the corresponding region of colicin B (73.0% identity, 12.5% similarity) (Fig. 5); they had 65.5% identity to the pore-forming domain of colicin A and 49.0% identity to the bacteriocidal domain of colicin N. Therefore, colicin U belongs to the group of pore-forming colicins (2, 15), which are characterized by a long hydrophobic sequence near the C terminus (38). This hydrophobic sequence, extending from residue 571 to 599, displays the lowest sequence similarity to the corresponding region of colicin B (Fig. 5) and therefore may determine the specificity of inactivation of colicin U by its cognate immunity protein. Colicins U and B displayed no cross-immunity.

The N-terminal sequence of colicin U shows the highest sequence similarity to bacteriocin 28b (Fig. 6), with 54.2% identity and an additional 16.0% similarity in the 130 N-terminal residues (27). A high degree of identity of the N-terminal sequences of colicins U and A (83.3%) was confined to the first 36 amino acid residues.

The putative receptor binding domain in the central region of colicin U revealed only low sequence similarity to that of other colicins. Nevertheless, significant sequence similarity between colicin U and colicin K (Fig. 7), which was restricted to a region ranging from residue 280 to 425 (34.6% identity), was observed. A higher sequence similarity (restricted to 44 residues) between colicin U and the TolA protein (Fig. 8), extending from residue 382 to 425 of the colicin U molecule (68.2% identity), was found. Short KAAAG stretches homologous to the KAAAD/E sequence, repeated in TolA, is contained once and only in colicin U. KA-rich regions occur also in other colicins.

Immunity protein of colicin U. The immunity protein of colicin U is homologous to the immunity proteins of pore-forming colicins, with the highest sequence similarity to colicin A (identity, 45.4%; similarity, 14.9%) and lower sequence simi-
Complex requirement of outer membrane determinants for colicin U entry into susceptible cells. Mutants of E. coli K-12 insensitive to colicin U were isolated from growth inhibition zones on plates onto which colicin U at a dilution titer of 10^6 was applied. Insensitive mutants isolated from the center of the inhibition zones, which contained the highest colicin concentration, were usually mutated in tol genes, as identified by their insensitivity to colicin E1, K, or L at the highest colicin concentrations available. Colonies isolated from the edge of the inhibition zone displayed a lower colicin U insensitivity (Table 2). These colonies carried mutations in ompA or ompF, as revealed by SDS-PAGE, which demonstrated the lack of OmpA or OmpF (data not shown). The patterns of resistance to colicin U of the mutants showed some overlap with the patterns of resistance to colicins A, K, L, and N, but no two mutants had identical resistance patterns (Table 2).

The colicin U sensitivity of multiple mutants from our strain collection was also tested. The sensitivity of strain HF24 ompF ompC was reduced 10^3-fold, and that of strain HO830 ompA ompF ompC was reduced 10^5-fold. Strain KS26-2 ompC ompF lamB and strain KO16 ompA ompF ompC lamB were fully resistant. The sensitivity of the porin mutants to colicin B remained unchanged, which indicated that resistance to colicin U did not result from a general disturbance of outer membrane integrity.

In addition, rfa mutants, which do not synthesize complete LPS but synthesize core LPS, were isolated from the colicin U inhibition zone, as shown by their sensitivity to bacteriophage C21 (45), to which the parent strain was resistant, and by silver staining after SDS-PAGE of isolated LPS (Fig. 9). An rfa mutant (HP77) had a 10-fold-reduced sensitivity to colicin U, an ompA mutant (DS1) displayed a 10^3-fold reduction in sensitivity, and an ompA rfa mutant (DS3) was resistant to colicin U (Table 2).

The killing by colicins U, K, and L of bacterial strains from various genera of the family Enterobacteriaceae that synthesize OmpA and of E. coli strains that synthesize OmpA proteins with defined point mutations is shown in Table 3. Colicin U displayed the same pattern of activity as colicin K, which indicated that the two colicins recognize a similar, if not identical, region of OmpA. In contrast, the sensitivity of these strains to colicin L differed from the sensitivity to colicin U and K (Table 2).

**TABLE 2. Sensitivities of ompA, ompF, and rfa mutants to colicins U, A, L, K, and N**

<table>
<thead>
<tr>
<th>E. coli straina</th>
<th>Plasmid genotype</th>
<th>Sensitivityb to colicin:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U</td>
</tr>
<tr>
<td>SK</td>
<td>Wild type</td>
<td>6</td>
</tr>
<tr>
<td>DS1</td>
<td>ompA</td>
<td>3</td>
</tr>
<tr>
<td>DS2</td>
<td>ompF</td>
<td>5</td>
</tr>
<tr>
<td>HP77</td>
<td>rfa</td>
<td>5</td>
</tr>
<tr>
<td>DS3</td>
<td>ompA rfa</td>
<td>r</td>
</tr>
<tr>
<td>F464</td>
<td>Wild type</td>
<td>5</td>
</tr>
<tr>
<td>F588</td>
<td>rfa</td>
<td>4</td>
</tr>
</tbody>
</table>

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* Strains DS1, DS2, DS3, and HP77 were isolated from the colicin U inhibition zone on plates seeded with E. coli K-12 SK. E. coli F464 and its derivatives F588 and F588 arose from E. coli O8:K27 (50).

b The numbers indicate the highest colicin dilutions active on bacteria (e.g., 5 = 10^5). r, resistant; —, not determined.
proteins (8, 10), as shown by the complete insensitivity of tolQ exbB and tolR exbBD mutants.

Deletion of 44 amino acid residues in the colicin U protein. To estimate the biological function of the region homologous to the TolA protein, the 44 amino acids of this region in colicin U were deleted, resulting in the production of ColUA382-425, which had the size expected (Fig. 3, lane 2). ColUA382-425 was as active as wild-type colicin U on E. coli 5K, and its toxicity to ompA, rfa, and tol mutant strains was decreased to the same extent as for wild-type colicin U. A colicin U-producing strain was as immune to ColUA382-425 as to wild-type colicin U (data not shown).

TABLE 3. Sensitivities to colicins U, K, and L of E. coli K-12 strains that synthesize different OmpA proteins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant phenotype</th>
<th>Sensitivity* to colicin:</th>
<th>U</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K-12</td>
<td>Wild type</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>UH99</td>
<td>Wild type</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>P400-2-2</td>
<td>OmpA (E68K)</td>
<td></td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>PS30-1cII</td>
<td>OmpA (V110D)</td>
<td></td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>UH100(a ompA-Sh)</td>
<td>OmpA from Shigella dysenteriae</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>UH100(a ompA-En)</td>
<td>OmpA from Enterobacter aerogenes</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>UH100(a ompA-Se)</td>
<td>OmpA from Serratia marcescens</td>
<td>0</td>
<td>0</td>
<td>t</td>
<td></td>
</tr>
</tbody>
</table>

* The numbers indicate the highest colicin dilutions active on bacteria (e.g., 5 = 10⁵). t, tolerant.

DISCUSSION

Colicin U is an addition to the list of bacteriocins (S1, S4, and J) found in Shigella strains (23, 52). The U colicinogenic serovars 1 and 8 of S. boydii display cross-immunity to each other and to the colicinogenic strains S. boydii Shp 132/56 (serovar 8) and S. boydii Shp 166/58 (serovar 1). Shigella is very similar to E. coli, and plasmids can easily be exchanged between the two genera, as has been found for Shigella sonnei, in which 70 colicin types have been identified, most of which are similar to E. coli (73% identity), colicin A (65.5% identity) (26), and colicin N (49% identity) assigns colicin U to the pore-forming colicins. The high sequence similarity is interrupted between residues 571 and 599; this region may be the specific recognition site of the colicin U immunity protein, which does not confer immunity to colicin B. In analogy to the X-ray structure of the pore-forming domain of colicin A, the region from residue 571 to 599 is located where the hydrophobic helix 8 extends into helix 9 (Fig. 5) (38, 53).

Uptake of colicin U and bacteriocin 28b is dependent on the tolA, -B, -Q, and -R genes. The N termini of the colicins have high sequence similarity (Fig. 6). Colicin U and colicin A contain the sequence DGTGWS, and bacteriocin 28 contains the sequence DGTNWS. These sequences are highly similar to the previously proposed TolA box DGSGS (39), which may be responsible for interaction of the translocation domain of group A colicins with TolA proteins.

TABLE 4. Binding of colicin U to E. coli 5K ompA, ompF, and rfa mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Adsorption (%)*</th>
<th>Sensitivity to colicin U t</th>
</tr>
</thead>
<tbody>
<tr>
<td>5K</td>
<td>Wild type</td>
<td>100 ± 14</td>
<td>6</td>
</tr>
<tr>
<td>Row</td>
<td>Wild type</td>
<td>100 ± 13</td>
<td>6</td>
</tr>
<tr>
<td>HP77</td>
<td>rfa</td>
<td>74 ± 7</td>
<td>5</td>
</tr>
<tr>
<td>DS2</td>
<td>ompF</td>
<td>72 ± 14</td>
<td>5</td>
</tr>
<tr>
<td>DS1</td>
<td>ompA</td>
<td>52 ± 4</td>
<td>3</td>
</tr>
<tr>
<td>DS3</td>
<td>ompA rfa</td>
<td>26 ± 12</td>
<td>r</td>
</tr>
</tbody>
</table>

* 95% confidence interval for the mean.

The numbers indicate the highest colicin dilutions active on bacteria (e.g., 6 = 10⁶). t, resistant.

TABLE 5. Sensitivity of tol and tonB mutants to colicin U

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Sensitivity to colicin U t</th>
</tr>
</thead>
<tbody>
<tr>
<td>5K</td>
<td>Wild type</td>
<td>6</td>
</tr>
<tr>
<td>BR158</td>
<td>tonB</td>
<td>6</td>
</tr>
<tr>
<td>CS5</td>
<td>tolC</td>
<td>6</td>
</tr>
<tr>
<td>A92</td>
<td>tolA</td>
<td>t</td>
</tr>
<tr>
<td>TPS13</td>
<td>tolQ</td>
<td>1</td>
</tr>
<tr>
<td>TPS300</td>
<td>tolR</td>
<td>2</td>
</tr>
<tr>
<td>A93</td>
<td>tolB</td>
<td>3</td>
</tr>
<tr>
<td>HE2</td>
<td>tolQ exbB</td>
<td>t</td>
</tr>
<tr>
<td>HE10</td>
<td>tolR exbBD</td>
<td>t</td>
</tr>
</tbody>
</table>

* The numbers indicate the highest colicin dilutions active on bacteria (e.g., 6 = 10⁶). t, tolerant.
assumed OmpF interaction sequence of colicin U and bacteriocin 28. Either the colicins interact with OmpF differently or the homologous sequence in colicin U and bacteriocin 28 specifies only interaction with the core LPS. The homologous sequence of 107 amino acids in the central part of colicins U and K may determine the identical requirement for OmpA, as revealed by the use of ompA mutants and OmpA proteins from different species. None of the single deletions in ompA and ompF conferred complete insensitivity to colicins U, A, L, K, and N; this supports previous findings that several outer membrane proteins are involved in the uptake of group A colicins, in contrast to group B colicins, for which deletion of single outer membrane proteins confers complete resistance.

The pore-forming group A colicins remain bound to the outer membrane receptors while they insert into the cytoplasmic membrane (4). This also seems to apply to group B pore-forming colicins, since the three-dimensional X-ray structure of colicin Ia reveals two very long α-helices, one of which connects the receptor binding domain with the pore-forming domain (61) and thus serves to bridge the periplasmic space. A very long α-helix in the TolA protein, required for the uptake of group A colicins, was proposed (35); this helix easily spans the periplasmic space from the cytoplasmic membrane to the outer membrane. Excision of a fragment extending from residue 68 to 209 of TolA does not inactivate the protein (19). Forty-four amino acids of colicin U showed a high sequence similarity to the central domain of the TolA protein; these amino acids could be excised (forming ColUA382-425) without inactivating colicin U. We did not find any changes in ColUA382-425 interaction with sensitive, tolerant, resistant, or immune bacterial cells, as compared with that of wild-type colicin U. Deletion of 35 amino acids in colicin A between residues 337 and 371 also caused no changes in the biological activity of colicin A (1). A repetitive Pro-X motif in the TonB protein is suggested to be involved in bridging the periplasmic space. Excision of the Pro-X motif (residues 66 to 100) in TonB slightly reduces TonB activity only under hyperosmolar conditions in which the periplasmic space is expanded (34). Although rigid structures designed to span the periplasmic space may be important for the action of certain colicins, TolA, and TonB, they are apparently not essential. It seems that there are sites of shorter distances between the outer membrane and cytoplasmic membrane, presumably fusion sites, through which TolA- and TonB-dependent colicins can enter cells, and TonB-dependent infection by certain phages and uptake of ferric siderophores and vitamin B12 might occur.

Colicin U-insensitive derivatives of E. coli K-12 that were not mutated in the tol genes were mutated in ompA, ompF, and rfa. Colicin U-resistant ompC and lamB mutants were not isolated in our experiments, even though existing mutants with these genotypes displayed a reduced sensitivity to colicin U. The porins can functionally substitute for each other, as shown by E. coli KS26-2 ompC ompF lamB, which is resistant to colicins U, L, A, K, and N. Binding of colicin N to the porins OmpF, OmpC, and PhoE has been demonstrated by microcalorimetry (22). The very low sensitivity of ompA deletion mutants to colicin U indicates a major role of OmpA in colicin U entry.

The immunity protein of colicin U (Cui) is 45% identical to that of colicin A (21), which explains the partial immunity of U coliogenic strains to colicin A. Although the sequence similarity between Cui and the colicin N immunity protein is much lower, Cui confers some immunity to colicin N. The immunity proteins of colicins U, B, A, and N exhibit a much higher sequence diversity than the pore-forming domains of the colicins with which they interact. The immunity proteins have to recognize the small differences between the pore-forming regions, implying selection for difference rather than similarity.

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