Plasmid **45,** 209–221 (2001)
doi:10.1006/plas.2001.1519, available online at http://www.academicpress.com on IDEAL®

Molecular Characterization of the Klebicin B Plasmid of *Klebsiella pneumoniae*

Margaret A. Riley, Theodora Pinou, John E. Wertz, Yin Tan, and Carla M. Valletta

Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut 06520

Received November 14, 2000; revised March 16, 2001

The nucleotide sequence of a bacteriocin-encoding plasmid isolated from *Klebsiella pneumoniae* (pKlebB-K17/80) has been determined. The encoded klebicin B protein is similar in sequence to the DNase pyocins and colicins, suggesting that klebicin B functions as a nonspecific endonuclease. The klebicin gene cluster, as well as the plasmid backbone, is a chimera, with regions similar to those of pore-former colicins, nuclease pyocins and colicins as well as noncolicinogenic plasmids. Similarities between pKlebB plasmid maintenance functions and those of the colicin E1 plasmid suggest that pKlebB is a member of the ColE1 plasmid replication family.

Key Words: bacteriocin; klebicin; evolution; colicin; plasmid.

Bacteriocins are the most abundant and diverse of the microbial defense systems. They are produced by all major groups of Bacteria and Archaea (Riley, 1998) (Dykes, 1995; Torreblanca *et al.*, 1989), are often produced at very high frequencies within a population, and exhibit extraordinary levels of protein diversity (Riley, 1998; Tagg *et al.*, 1976). In *Escherichia coli*, bacteriocins are exclusively encoded on plasmid replicons.

The colicins of *E. coli* have served as a model system for investigating the evolutionary mechanisms involved in bacteriocin diversification (Riley, 1993a, 1993b, 1998; Riley and Gordon, 1995; Pilsl *et al.*, 1999; Tan and Riley, 1995, 1997; Braun *et al.*, 1994; Roos *et al.*, 1989). Phylogenetic studies distinguish two major colicin families, the pore-former and nuclease colicins (Riley, 1998). These two families appear to differ in their modes of diversification. The pore-former colicins, which kill by forming channels in the cytoplasmic membrane, are a relatively ancient family that has diversified primarily through the action of recombination (reviewed in Riley, 1998). The nuclease colicins are a more recent family of proteins that kill by nonspecific digestion of DNA or specific cleavage of RNA (James *et al.*, 1991). The nuclease colicins investigated have diversified primarily through positive selection acting on point mutations that result in novel immunity phenotypes (reviewed in Riley, 1998).

Two questions arise from studies of colicin evolution. First, why do the two families of colicins experience such different evolutionary forces, as described above? Second, are the patterns of sequence divergence observed for *E. coli* plasmid-encoded colicins representative of bacteriocin evolution? We provide an evolutionary investigation of a bacteriocin plasmid isolated from *Klebsiella pneumoniae* that sheds light on both of these issues.

The bacteriocin plasmid pKlebB-K17/80 (pKlebB) was isolated from *K. pneumoniae* and identified as a non-self-transmissible plasmid encoding a bacteriocin-like killing phenotype (James, 1988). James further localized the bacteriocin gene cluster using a combination of subcloning and transposon mutagenesis. From restriction site analysis and mapping studies, James noted several structural and organizational similarities between the bacteriocin gene cluster of klebicin B and those of the E-colicins of *E. coli*.

We report here the nucleotide sequence of the entire pKlebB plasmid. The location of the bacteriocin gene cluster was inferred from DNA and protein sequence comparisons with all characterized bacteriocins. These comparisons suggest that klebicin B functions as a DNase bacte-

0147-619X/01 $35.00

riocin. Further, the klebicin B plasmid is shown to be a chimera, composed of regions similar to those of several members of the colicin plasmid family of *E coli*, the chromosomally encoded nuclease pyocins of *Psuedomonas aeruginosa* and several additional ColE1-like plasmids isolated from *Klebsiella oxytoca* and *K. pneumoniae*. The klebicin B plasmid provides one of the first indications that recombinational diversification is not restricted to the pore-former bacteriocins.

MATERIALS AND METHODS

Plasmid

Richard James kindly provided the plasmid pRJ180. This plasmid was constructed by inserting pKlebB-K17/80 into the *Hin*dIII site of pUC19 (James, 1988).

DNA Preparation and Sequencing

pRJ180 was transformed into competent DH5α *E. coli* cells (Life Technologies Inc., Gaithersburg, MD) using standard methods, and ampicillin-resistant colonies were selected on LB agar containing ampicillin (50 μg/ml) (Ausubel *et al.*, 1992). Cells were grown overnight in 5 ml of LB broth containing ampicillin (50 μg/ml) at 37°C, and plasmid DNA was isolated using a QIAprep kit (Qiagen, Chatsworth, CA).

Approximately 300 ng of plasmid DNA was used for cycle sequencing according to the protocol of the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer, Palo Alto, CA). Primers were designed to anneal to the pUC19 vector and were employed to initiate DNA sequencing. Additional primers were constructed at approximately 250- to 300-bp intervals. Primer locations and sequences are available upon request. The nucleotide sequence of the plasmid pKlebB-K17/80 is available from GeneBank (Accession No. AF156893).

Computer Analysis

DNA sequence data were assembled and edited with DNASTAR programs (DNASTAR Inc., Madison, WI). The nucleotide and protein sequences were submitted to the National Center for Biological Information and the BLAST (Altschul *et al.*, 1990) network service was employed to search for similar sequences in the database.

Nucleotide and protein sequence alignments were made with the Clustal-W algorithm of the LASERGENE program (DNASTAR Inc., 1999). Gene trees were inferred using neighbor-joining and maximum likelihood and parsimony algorithms (Saitou and Nei, 1987; Swofford, 1993). Heuristic searches were used. Gaps were not treated as characters in any analysis. All methods produced a single, congruent tree for each gene, with the exception of the maximum parsimony applied to the nuclease region tree. In that case, two trees were found and a consensus tree was compiled. Figures 2 and 3 represent gene trees inferred with a maximum likelihood model. Figures 4 and 7 report gene trees inferred with a maximum parsimony model. Confidence in the inferred tree topologies was assessed by application of a bootstrap, with 1000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

Klebicin B Plasmid

The entire pKlebB-K17/80 DNA sequence was determined. The plasmid is 5258 base pairs and encodes seven recognizable functions. Figure 1 illustrates the organization of the pKlebB plasmid and Table 1 provides the position of each predicted function.

Bacteriocin Gene Cluster

Three bacteriocin-related genes (klebicin, immunity, and lysis) were detected as a cluster (Fig. 1). The three genes are oriented in the same direction, a pattern typical for nuclease colicin gene clusters.

The klebicin B lysis gene is 156 bp and encodes a protein of 51 amino acids with an estimated molecular weight of 5 kDa (Fig. 1 and Table 1). Lysis proteins are involved in the release of bacteriocin from the producing cell. All characterized lysis genes were aligned and the gene tree inferred is shown in Fig. 2. The klebicin B lysis gene is most similar to the lysis genes of colicin gene clusters A and S4, two

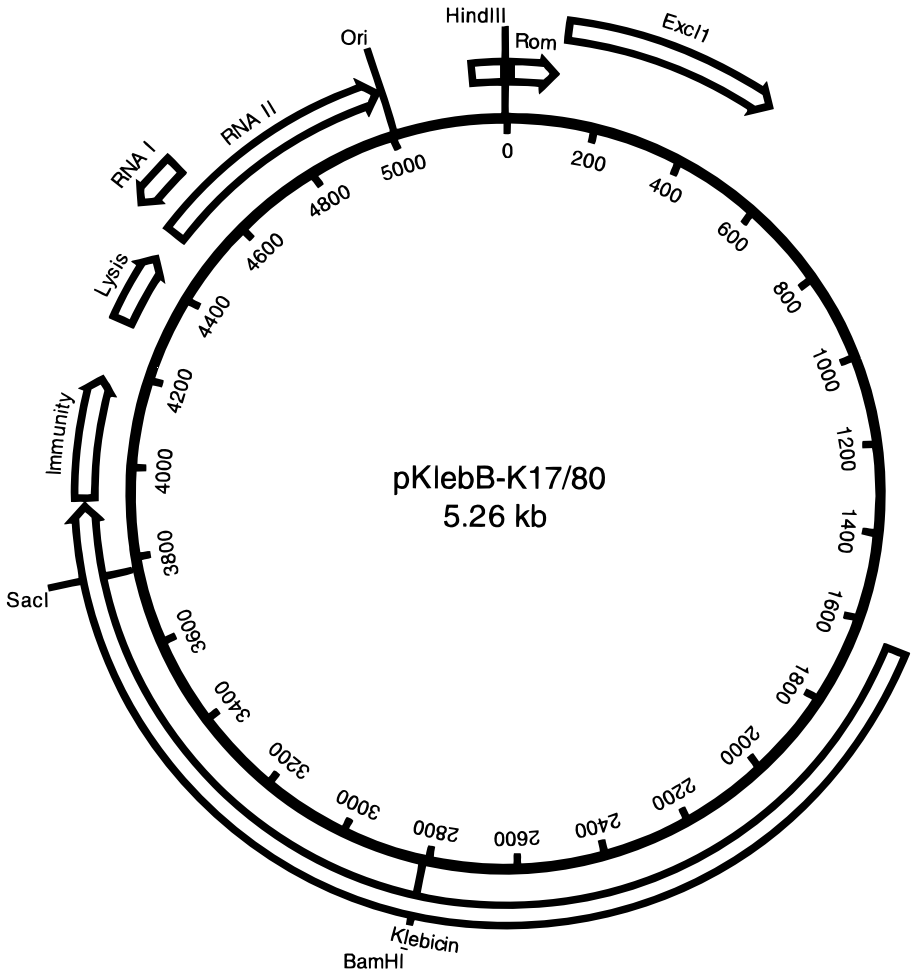

FIG. 1. Physical and genetic map of pKlebB-k17/80. The arrows represent functional regions detected by DNA and protein sequence comparisons and the inferred direction of gene expression. The origin of replication is indicated by a vertical line, labeled Ori. Several restriction endonuclease sites are indicated.

TABLE 1

Nucleotide Positions of Inferred Functional Regions of pKlebB-K17/80

Gene	Location (bp)
Klebicin	1628–3925
Immunity	3927–4184
Lysis	4292–4447
RNA I	4601–4496
RNA II	4494–5001
Ori	5002
Rom	5181–105
Excll	105–506

members of the colicin pore-former family isolated from *Citrobacter freundii* (A) (Morlon, 1983) and *E. coli* (S4) (Pilsl *et al.*, 1999). Levels of lysis protein sequence identity are 61 and 62% between klebicin B and colicins A and S4, respectively.

The lysis and immunity genes are separated by 107 bp (Fig. 1). This region is most similar to the corresponding region of the colicin A and S4 gene clusters, with 50 and 41% DNA sequence identity, respectively. The 3′ flanking region (110 bp) of the klebicin B lysis gene is also most similar to the corresponding region of the col-

icin A and S4 gene clusters, with 89 and 92% DNA sequence identity, respectively.

The klebicin B immunity gene is 258 bp and encodes a protein of 85 amino acids with an estimated molecular weight of 9.6 kDa. A single bp separates the 5′ end of the immunity gene from the 3′ end of the klebicin gene (Fig. 1). Immunity proteins interact with the C-terminal region of their corresponding bacteriocin proteins, conferring immunity to the host cell or other cells encoding the same bacteriocin. Sequence comparisons of the klebicin B immunity protein with all characterized immunity proteins reveal low levels of sequence similarity, save for those associated with the family of DNase colicins of *E. coli* (E2, E7–E9) and the pyocins of *P. aeruginosa* (S1, S2, and AP41).

Immunity gene sequences were aligned for this subset of sequences and the inferred gene tree is provided in Fig. 3. The klebicin B immunity gene is most similar to the pyocin immunity genes. The topology of this tree is not well supported by bootstrap analysis, implying that these immunity protein sequences are too divergent to

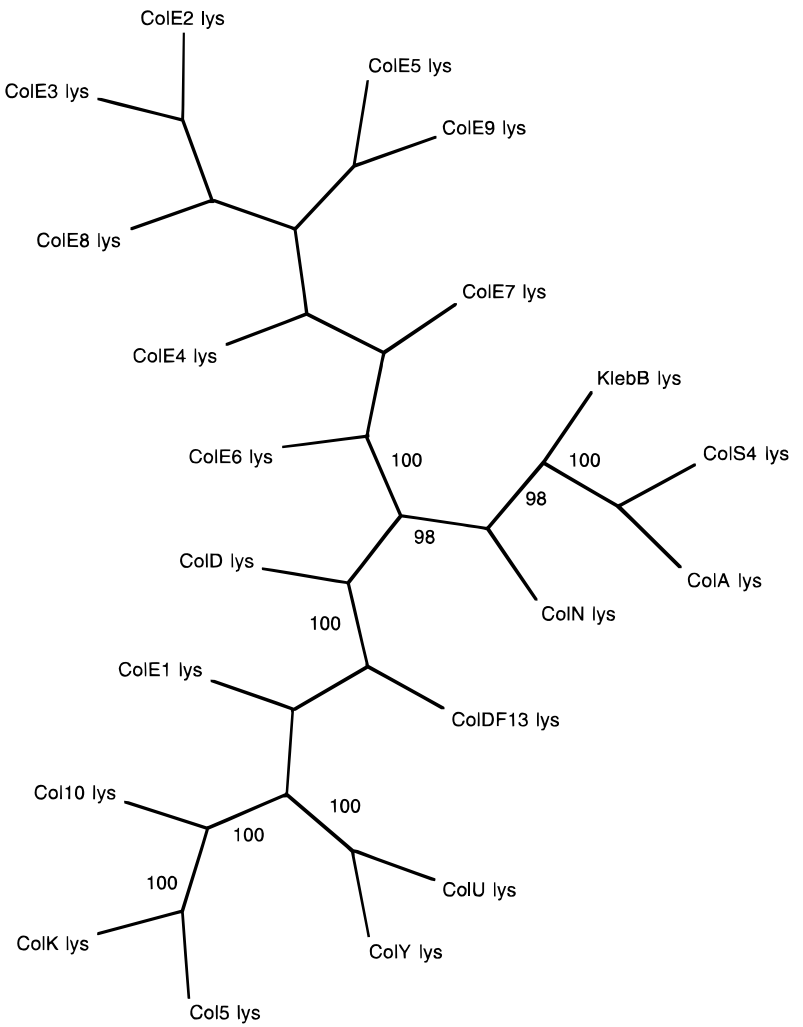

FIG. 2. A maximum likelihood network inferred for lysis DNA sequences. Bootstrap values above 80% are indicated on the appropriate branches.

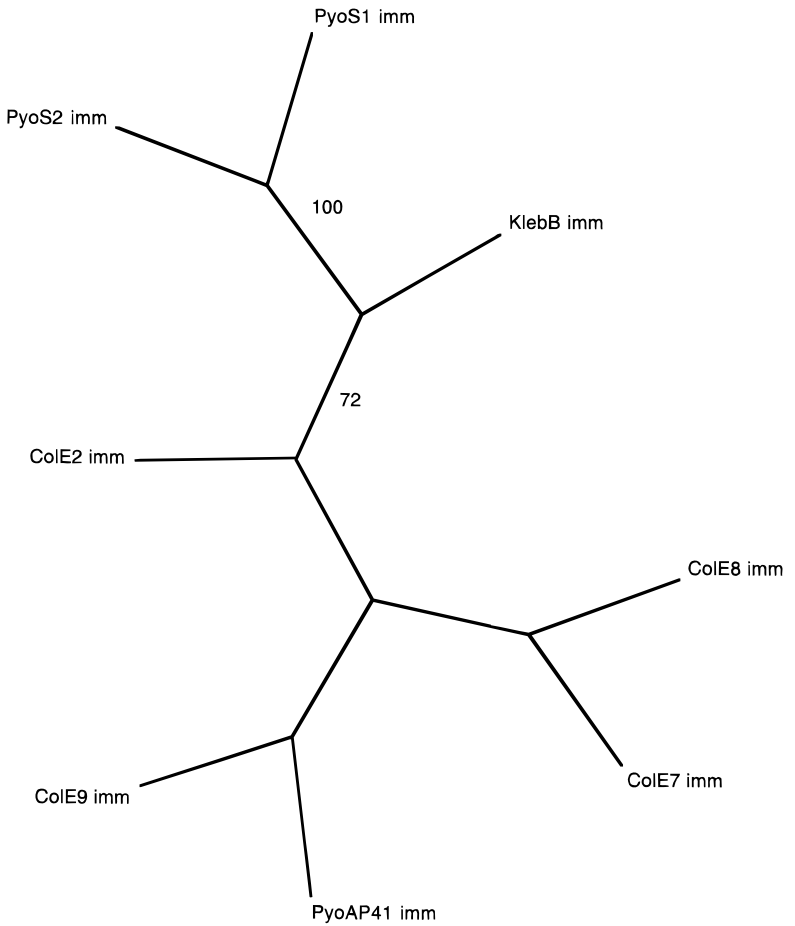

FIG. 3. A maximum likelihood network inferred for immunity DNA sequences. Bootstrap values above 70% are indicated on the appropriate branches.

allow precise reconstruction of their ancestral relationships. The klebicin B immunity protein is most similar to the immunity proteins of pyocins S1 and S2 with 57% protein sequence identity. It is slightly less similar to the immunity proteins of colicins E2 and E7–E9, with 56, 52, 51, and 52% sequence identity, respectively. The klebicin B immunity protein shows no detectable similarity to colicins A and S4.

As noted above, the region 3′ to the immunity gene is most similar in sequence to the corresponding region of the colicin A and S4 gene clusters. This region could not be aligned between klebicin B and the nuclease pyocins or colicins.

The klebicin B bacteriocin gene is predicted to be 2298 bp and encodes a protein of 765 amino acids. The estimated molecular weight of klebicin B is 79.7 kDa, which agrees with that reported by James (1988). Bacteriocin proteins contain at least four functional domains: cell surface receptor recognition, translocation across the cell membrane, cell killing, and immunity protein binding (reviewed in James *et al.*, 1996). In colicins, these functions are ordered as follows: translocation functions are found in the N-terminal portion of the protein, receptor recognition comprises the middle of the protein, and the killing domain and immunity binding region are located in the carboxy-

terminal (C-terminal) region (James *et al.*, 1996).

A BLAST search reveals similarity with other bacteriocin proteins restricted to the C-terminal half of the klebicin B protein. The most similar bacteriocins with respect to this region are the DNase colicins (E2, E7–E9) and pyocins (S1, S2, and AP41). Levels of protein sequence identity with the final 132 amino acid residues of klebicin B range from 43 (S1) to 29% (E9). Figure 4 provides a gene tree inferred for the C-terminal regions of the DNase bacteriocins. Klebicin B is intermediate in position between the DNase colicins and pyocins.

The DNA sequence immediately 5′ to the klebicin B bacteriocin gene encodes a set of regulatory elements. All colicin gene clusters whose regulatory regions have been sequenced contain a σ^{70} promoter, a LexA binding site, and a Shine-Dalgarno box (Fig. 5). The klebicin B

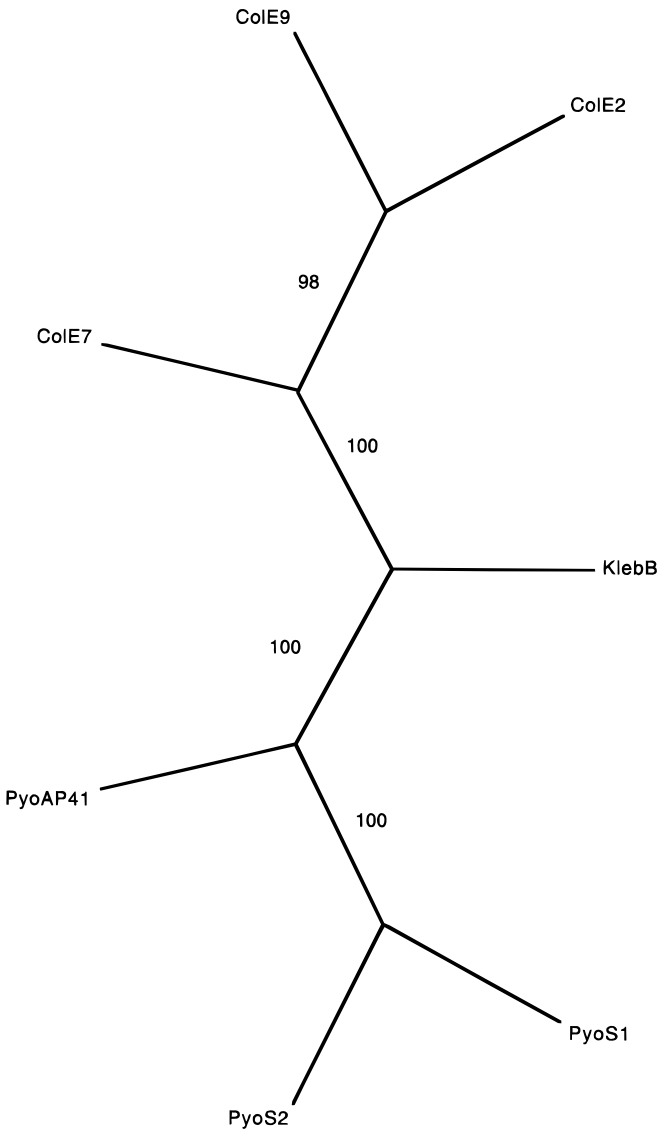

FIG. 4. A maximum parsimony network inferred for the C-terminal half of the nuclease colicins, pyocins, and klebicin B. Bootstrap values above 80% are indicated on the appropriate branches.

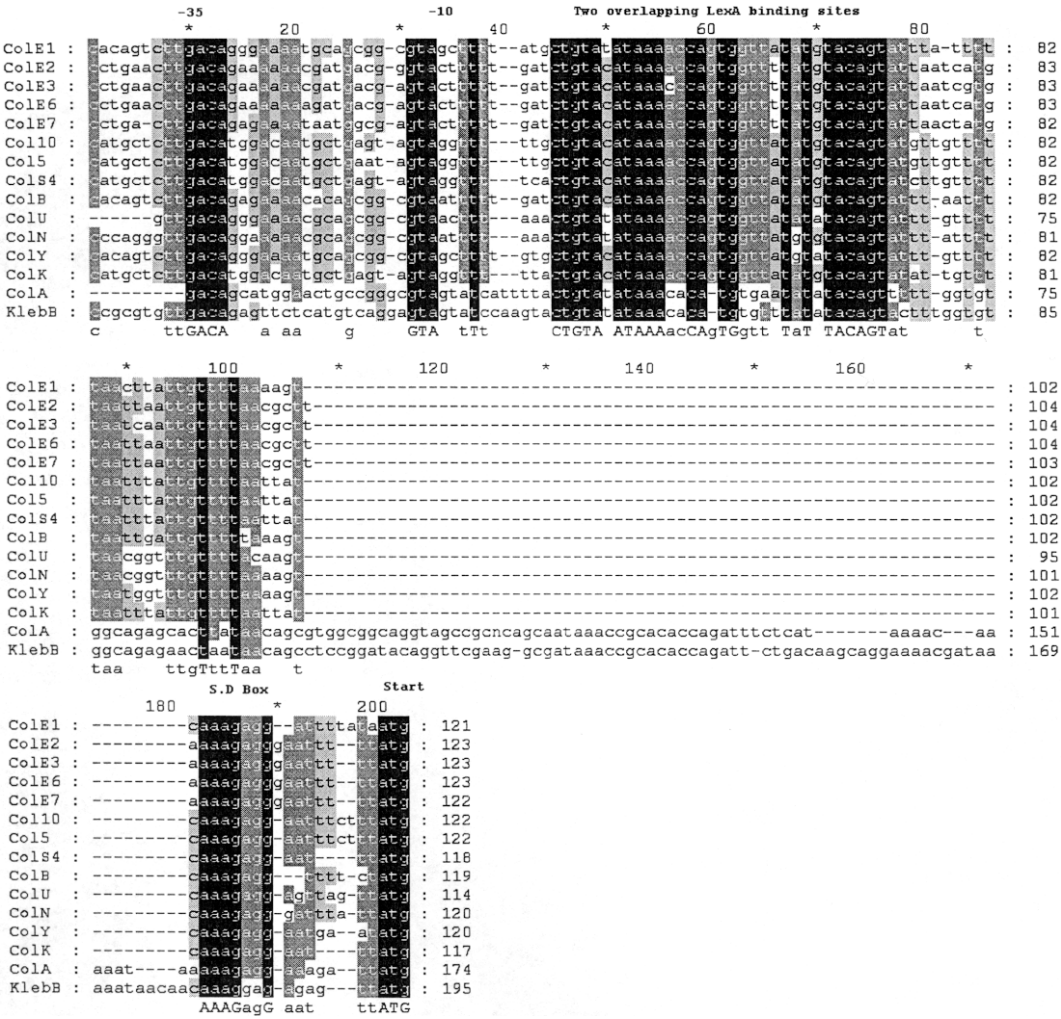

FIG. 5. An alignment of the regulatory regions of bacteriocin gene clusters annotated to show identified regulatory motifs. Sequences are from ColE1 (Tomizawa, *et al.*, 1977), ColE2 (Cole *et al.*, 1985), ColE3 (Masaki and Ohta, 1985), ColE6 (Akutsu *et al.*, 1989), ColE7 (Soong *et al.*, 1992), Col10 (Pilsl and Braun, 1995a), Col5 (Pilsl and Braun, 1995b), ColS4 (Pilsl *et al.*, 1999), ColB (Schramm *et al.*, 1987), ColU (Smajs *et al.*, 1997), ColN (Pugsley, 1987), ColY (Riley, 2000), ColK (Kuhar and Zgur-Bertok, 1999), ColA (Morlon *et al.*, 1983), and KlebB (this work). Asterisks designate 10 unit intervals.

gene cluster contains all of these common elements and shares high levels of sequence similarity throughout this control region with those associated with the characterized colicin gene clusters. σ^{70} promoter sequences of colicin gene clusters are highly conserved; in fact, the −35 sequences exactly match the consensus sequence. All colicin gene clusters contain a LexA binding site 3–4 bases downstream of the Pribnow box. The LexA binding site is actually composed of two overlapping binding sites, with the exception of colicin gene clusters for Ia and Ib (Mankovich *et al.*, 1986), which have a single site.

The colicin A gene cluster shows significant deviation from the typical colicin regulatory region relative to the majority of other colicin gene clusters. The colicin A gene cluster has an inser-

tion of about 60 bp located between the double LexA binding site and the Shine-Dalgarno box. The colicin A gene cluster regulatory region also lacks the highly conserved thymine-rich region of unknown function found just downstream of the LexA binding site (at nucleotide positions 87–100, Fig. 5).

Overall, the klebicin B gene cluster regulatory region most closely resembles that of colicin A. It contains a slightly larger insert (~72 bp), it lacks the thymine-rich region conserved in all other colicin regulatory regions (Fig. 5), and it shares the highest degree of sequence identity (69%) with the Col A gene cluster regulatory region.

Klebicin B Killing Function

The DNase killing domain is contained within a short stretch (132 aa) of the C-terminal regions of DNase colicins and pyocins (James *et al.*, 1991; Sano *et al.*, 1990). An alignment of this killing domain among all nuclease colicins, pyocins, and klebicin B reveals that the DNase and RNase colicins share little detectable sequence similarity. Five residues (amino acid positions 10, 44, 101, 125, and 126; Fig. 6) are shared by all nuclease colicins and an additional four residues (amino acid positions 47, 52, 83, and 97; Fig. 6) are shared by all but one member of the entire nuclease group. In contrast, 37 residues are shared by all DNase colicins, pyocins and klebicin B and an additional 20 residues are shared by all but one member of the DNase group. The RNase colicins are most similar in this region, sharing all but 21 of the 132 residues.

Klebicin B is identical to the consensus sequence of the DNase killing domain at all but 7 of the 44 sites shared by other members of the DNase group (Fig. 6). Such high levels of sequence similarity argue that klebicin B is a member of the nonspecific endonuclease family of nuclease bacteriocins. A gene tree inferred for the nuclease region clearly distinguishes the RNase and DNase bacteriocins (Fig. 7) and reveals that klebicin B clusters within the DNase branch of this tree and shares greater similarity with the nuclease pyocins of *P. aeruginosa*.

There is no detectable sequence similarity between the N-terminal region of klebicin B and those of any other bacteriocin proteins, suggesting that klebicin B has unique receptor recognition and translocation systems.

Klebicin B Plasmid Maintenance Functions

BLAST searches reveal several regions that are involved in pKlebB maintenance, such as replication control and mobilization. These functions were inferred from sequence similarity with the corresponding regions of several colicin plasmids, including pCol A (Zverev and Khmel, 1985), pCol E1 (Chan *et al.*, 1985), pClo DF13 (Nijkamp *et al.*, 1986), pCol Y (Riley *et al.*, 2000) and pBERT from *Salmonella bertii* (Hanes *et al.*, unpublished), and nonbacteriocin plasmids from *Klebsiella* and *Salmonella*, *K. pneumoniae* plasmid pJHCMWI (Dery *et al.*, 1997), *K. oxytoca* pNBL63 (Wu, 1999) and pTKHII (Wu, 1999), and *Salmonella typhimurium* cryptic plasmid pIMVSI (Astill, 1993).

pKlebB appears to replicate in the same way as ColE1 (Chan *et al.*, 1985). This form of replication is mediated by chromosomally encoded proteins, and the frequency of initiation of replication is regulated by two plasmid-derived transcripts, an RNA primer, RNA II, and a modulating antisense RNA, RNA I, encoded upstream of the point of initiation of DNA synthesis (Polisky, 1988). Some plasmids using this type of replicon also encode a small protein (Rom or Rop) involved in the modulation of the initiation process (Mikiewicz, 1997).

The antisense RNA I and RNA II region of pKlebB (Fig. 1 and Table 1), although similar to all ColE1-type plasmids, is most closely related to the corresponding region of pJHCMWI (isolated from *K. pneumoniae*), pIMVSI (isolated from *S. typhimurium*), and p15A (isolated from *E. coli*). The inferred secondary structure of RNA I from pKlebB has the three hairpin loops characteristic of ColE1-type plasmids (typically designated I′, II′, and III′) (Tomizawa and Itoh, 1981). Most mutations, which result in altered incompatibility, are located in loops I′ and II′ (Davison, 1984). The RNA I sequences from p15A (Selzer, 1983), pJHCMWI, and pIMVSI

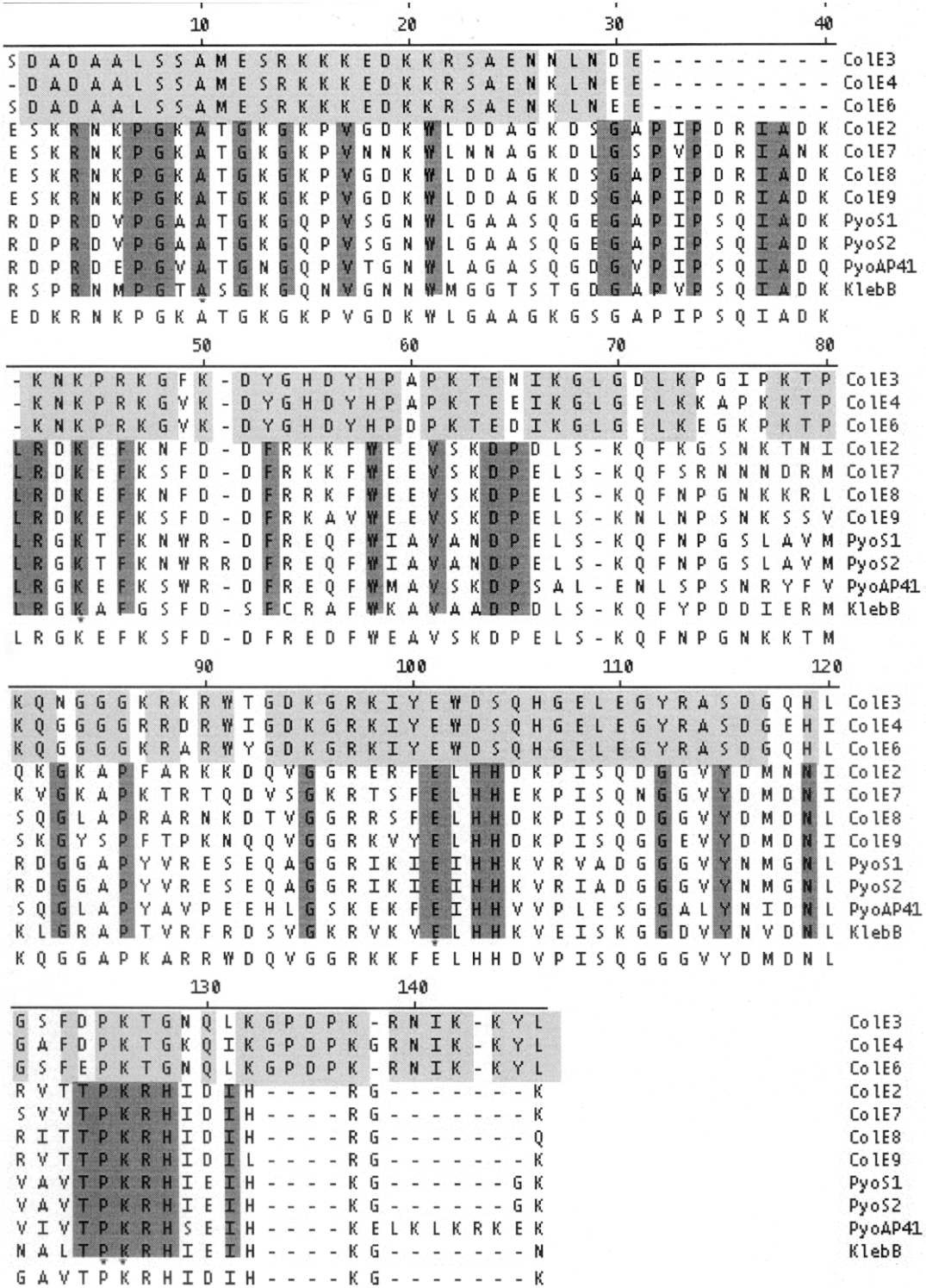

FIG. 6. An alignment of the C-terminal 132 amino acids of the nuclease colicins, pyocins, and klebicin B. Gray shading indicates those residues conserved among all RNase colicins. Dark gray shading indicates those residues shared among all DNase colicins. Stars indicate those residues shared by all nuclease colicins, pyocins, and klebcin B.

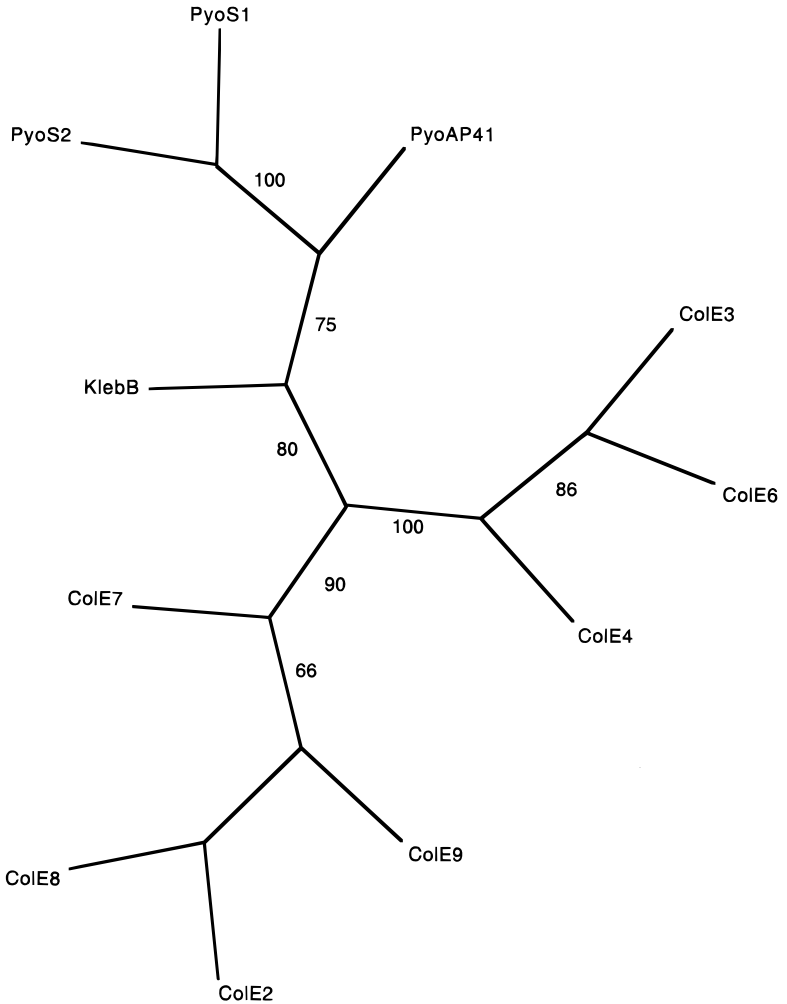

FIG. 7. A maximum parsimony network inferred for the C-terminal 132 amino acids of the nuclease colicins, pyocins, and klebicin B. Bootstrap values above 60% are indicated on the appropriate branches.

are all more similar to each other (2–5 substitutions in the region containing secondary structure) than they are to pKlebB (10–12 substitutions and two insertions in the same region). The majority of sequence variations between the KlebB sequence and the other RNA I sequences occurs in loops I′ and II′ (one insertion and 6–8 substitutions). Since pJHCMWI and p15A are compatible (Dery *et al.*, 1997) and differ by 5 substitutions, only one of which is in loop I′, it is reasonable to assume that pKlebB would be compatible with the other three plasmids.

The pKlebB Rom protein (Fig. 1 and Table 1) is most similar to the Rom protein encoded by plasmid pNBL63 isolated from *K. oxytoca*. These two Rom proteins differ at only two sites. Mikiewicz *et al.* (1997) proposed that a family of Rom proteins existed, based upon the sequence similarity observed among three characterized Rom proteins. Additional Rom proteins have been described and a comparison of these proteins firmly establishes the existence of this protein family (Riley, data not shown).

A region similar in sequence to the exclusion region of ColE1 is also detected in pKlebB. BLAST searches reveal that the encoded Exc1 protein is most similar to that found in pNBL63. The Exc1 proteins of pKlebB and pNBL63 are quite similar to each other and highly divergent from the corresponding protein in ColE1, with 95% sequence identity between pKlebB and pNBL63 and 27% sequence identity between pKlebB and ColE1. In ColE1 this region encodes two proteins (Exc1 and Exc2), which are involved in a form of plasmid incompatibility known as entry exclusion (Chan *et al.*, 1985). The mechanism of incompatibility involves the inhibition of conjugal transfer of a plasmid into a host cell. A second Exc protein was not detected in either pKlebB or pNBL63.

Recombination in pKlebB

pKlebB is a chimeric plasmid. Figure 8 illustrates the regions of pKlebB that differ in sequence origin. Based upon DNA and protein sequence comparisons, we deduce that the bacteriocin gene cluster is the product of at least three recombination events that have joined a pore-former type of 5′ regulatory region and lysis gene (most similar to colicin A), a DNase type of bacteriocin and immunity region (most similar to pyocin S1 and colicin E9), and a receptor recognition and translocation region of unknown origin. The plasmid "backbone," which is composed of plasmid maintenance functions, also based on sequence comparison, appears to be the product of recombination events that have joined a *K. pneumoniae* RNA I and RNA II region (most similar to pJHCMWI) and a *K. oxytoca* Rom and Exc1 region (most similar to pNBL63). Although this region bears the signature ColE1-like replication functions, levels of sequence similarity suggest that it is a *Klebsiella*-specific plasmid backbone.

Recombination has been implicated in the origin and evolution of numerous native plasmids. Indeed, it has been argued that plasmids are merely shuttle vectors that serve to transport genes from one chromosomal background to another. Thus, it was surprising to find a class of plasmids, the nuclease colicin plasmids of *E. coli*, in which recombination appears to have played little or no role in their origin and diversification (Riley, 1993a). In fact, nuclease colicins and their plasmids were shown to diversify primarily through the action of positive selection on mutations that result in novel immunity functions (reviewed in Riley, 1998). pKlebB is the first example of a plasmid-encoded nuclease bacteriocin originating from multiple recombination events.

It is possible that a two-step process is involved in nuclease bacteriocin plasmid evolution. Recombination may serve in the creation of novel plasmids that combine bacteriocin functions from multiple ancestries, such that appropriate receptor recognition functions are combined with existing killing and immunity

FIG. 8. The chimeric nature of the pKlebB sequence is indicated by alternate shadings. The key indicates the probable ancestry of each region.

functions and these are coupled with a replicon that can survive in a particular chromosomal background. Those recombinants that survive may then experience the force of strong positive, diversifying selection detected for the nuclease colicins as they subsequently diversify within that species.

A prediction of this two-step hypothesis is that nuclease bacteriocins within a species will be closely related and show the pattern of sequence divergence typical of diversifying selection [as observed for the nuclease colicin plasmids (Riley, 1998)]. When compared between species, the chimeric origins of these same bacteriocins will be revealed. With respect to the nuclease bacteriocins of *Klebsiella*, this question can only be addressed with the characterization of additional nuclease klebicin plasmids. Analysis of pKlebB has revealed that the plasmid-encoded nuclease bacteriocins are likely created by recombination, as was seen for the pore-former colicins of *E. coli*. How the family of nuclease klebicins diversifies remains to be addressed.

ACKNOWLEDGMENTS

We thank Richard James for supplying the klebicin B plasmid and acknowledge support from the NIH and NSF (GM58433 and DEB-9458247).

REFERENCES

Akutsu, A., Masaki, H., and Ohta, T. (1989). Molecular structure and immunity specificity of colicin E6, an evolutionary intermediate between E-group colicins and cloacin DF13. *J. Bacteriol.* **171** (12), 6430–6436.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215,** 403–410.

Astill, D., Manning, P., and Heuzenroeder, M. (1993). Characterization of the small cryptic plasmid, pIMVS1 of *Salmonella enterica ser. Typhimurium. Plasmid* **30,** 258–267, doi:10.1006/plas.1993.1057.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1992). "Current Protocols in Molecular Biology." J. Wiley, New York.

Braun, V., Pilsl, H., and Groß, P. (1994). Colicins: Structures, modes of actions, transfer through membranes, and evolution. *Arch. Microbiol.* **161,** 199–206.

Chan, P. T., Ohmor, H., Tomizawa, J., and Lebowitz, J. (1985). Nucleotide Sequence and Gene Organization of colE1 DNA*. *J. Biol. Chem.* **260** (15), 8925–8935.

Cole, S. T., Saint-Joanis, B., and Pugsley, A. P. (1985). Molecular characterisation of the colicin E2 operon and idenification of its products. *Mol. Gen. Genet.* **198,** 465–472.

Davison, J. (1984). Mechanism of control of DNA replication and incompatibility in ColE1-type plasmids—A review. *Gene* **28,** 1–15.

Dery, K. J., Chavideh, R., Waters, V., Chamorro, R., Tolmasky, L., and Tolmasky, M. (1997). Characterization of the replication and mobilization regions of the multiresistance *Klebsiella pneumoniae* plasmid pJHCMW1. *Plasmid* **38,** 97–105, doi:10.1006/plas.1997.1303.

DNASTAR Inc. (1999). "LASERGENE," Fourth Ed. DNASTAR Inc., Madison, WI.

Dykes, C. A. (1995). Bacteriocins: Ecological and evolutionary significance. *Trends Ecol. Evol.* **10,** 186–189.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39,** 783–791.

James, R. (1988). Molecular cloning and purification of klebicin B. *J. Gen. Microbiol.* **134,** 2525–2533.

James, R., Kleanthous, C., and Moore, G. R. (1996). The biology of E colicins: Paradigms and paradoxes. *Microbiology* **142,** 1569–1580.

James, R., Lazdunski, C., and Pattus, F., (1991). "Bacteriocins, Microcins, and Lantibiotics," Vol. 65, NATO ASI series. Springer-Verlag, New York.

Kuhar, I., and Zgur-Bertok, D. (1999). Transcription regulation of the colicin K *cka* gene reveals induction of colicin synthesis by differential responses to environmental signals. *J. Bacteriol.* **181** (23), 7373–7380.

Mankovich, J. A., Hsu, C.-H., and Konisky, J. (1986). DNA and amino acid sequence analysis of structural and immunity genes of colicins Ia and Ib. *J. Bacteriol.* **168** (1), 228–236.

Masaki, H., and Ohta, T. (1985). Colicin E3 and its immunity genes. *J. Mol. Biol.* **182,** 217–227.

Mikiewicz, D., Wrobel, B., Wegrzyn, G., and Plucienniczak, A. (1997). Isolation and characterization of a Col E1-like plasmid from *Enterobacter agglomerans* with a novel variant of rom gene. *Plasmid* **38,** 210–219, doi:10.1006/plas.1997.1312.

Morlon, J., Lloubes, R., Varenne, S., Chartier, M., and Lazdunski, C. (1983). Complete nucleotide sequence of the structural gene for colicin A, a gene translated at a non-uniform rate. *J. Mol. Biol.* **170,** 271–285.

Nijkamp, H., deLang, R., Stuitje, A., Elzen, P. v. d., Veltkamp, E., and Putten, A. v. (1986). The complete nucleotide sequence of the bacteriocinogenic plasmid CloDF13. *Plasmid* **16,** 135–160.

Pilsl, H., and Braun, V. (1995a). Novel colicin 10: Assignment of four domains to TonB- and TonC-dependent uptake via the Tsx receptor and to pore formation. *Mol. Microbiol.* **16** (1), 57–67.

Pilsl, H., and Braun, V. (1995b). Strong function-related homology between the pore-forming colicins K and 5. *J. Bacteriol.* **177** (23), 6973–6977.

Pilsl, H., Smajs, D., and Braun, V. (1999). Characterization of colicin S4 and its receptor, OmpW, a minor protein of the *Escherichia coli* outer membrane. *J. Bacteriol.* **181** (11), 3578–81.

Polisky, B. (1988). ColE1 replication control circuitry: Sense from antisense. *Cell* **55** (6), 929–32.

Pugsley, A. P. (1987). Nucleotide sequencing of the structural gene for colicin N reveals homology between the catalytic, C-terminal domains of colicins A and N. *Mol. Microbiol.* **1** (3), 317–325.

Riley, M., Cadavid, L., Collett, M., Neely, M., Adams, M., Phillips, C., Neel, J., and Friedman, D. (2000). The newly characterized colicin Y provides evidence of positive selection in pore-former colicin diversification. *Microbiology* **146,** 1671–1677.

Riley, M. A. (1993a). Molecular mechanisms of colicin evolution. *Mol. Biol. Evol.* **10,** 1380–1395.

Riley, M. A. (1993b). Positive selection for colicin diversity in bacteria. *Mol. Biol. Evol.* **10,** 1048–1059.

Riley, M. A. (1998). Molecular mechanisms of bacteriocin evolution. *Ann. Rev. Genet.* **32,** 255–278.

Riley, M. A., and Gordon, D. (1995). Ecology and evolution of bacteriocins. *J. Ind. Microbiol.* **17,** 155–158.

Roos, U., Harkness, R. E., and Braun, V. (1989). Assembly of colicin genes from a few DNA fragments: Nucleotide sequence of colicin D. *Mol. Microbiol.* **3** (7), 891–902.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4** (4), 406–425.

Sano, Y., Matsui, H., Kobayashi, M., and Kageyama, M. (1990). Pyocins S1 and S2, bacteriocins of *Pseudomonas aeruginosa*. In "*Pseudomonas:* Biotransformations, Pathogenesis, and Evolving Biotechnology" S., Silver, Ed.), pp. 352–358. Am. Soc. Microbiology, Washington, DC.

Schramm, E., Mende, J., Braun, V., and Kamp, R. M. (1987). Nucleotide sequence of the colicin B activity gene cba: Consensus pentapeptide among TonB-dependent colicins and receptors. *J. Bacteriol.* **169** (7), 3350–3357.

Selzer, G., Som, T., Itoh, T., and Tomizawa, J. (1983). The origin of replication of plasmid p15A and comparative studies on the nucleotide sequences around the origin of related plasmids. *Cell* **32,** 119–129.

Smajs, D., Pilsl, H., and Braun, V. (1997). Colicin U, a novel colicin produced by *Shigella boydii*. *J. Bacteriol.* **179** (15), 4919–4928.

Soong, B., Lu, F., and Chak, K. (1992). Characterization of the *cae* gene of the ColE7 plasmid. *Mol. Gen. Genet.* **233,** 177–183.

Swofford, D. L. (1993). "PAUP: Phylogenetic Analysis Using Parsimony," Version 3.01. Champaign, IL.

Tagg, J. R., Dajani, A. S., and Wannamaker, L. W. (1976). Bacteriocins of gram positive bacteria. *Bacterial Rev.* **40,** 722–756.

Tan, Y., and Riley, M. A. (1995). Rapid invasion of colicinogenic bacteria with novel immunity functions. *Microbiology* **142,** 2175–2180.

Tan, Y., and Riley, M. A. (1997). Nucleotide polymorphism in colicin E2 gene clusters: Evidence for nonneutral evolution. *Mol. Biol. Evol.* **14,** (6), 666–673.

Tomizawa, J. I., and Itoh, T. (1981). Plasmid ColE1 incompatibility determined by interaction of RNA I with primer transcript. *Proc. Natl. Acad. Sci. USA* **78,** 6096–6100.

Tomizawa, J. I., Ohmori, H., and Bird, R. E. (1977). Origin of replication of colicin E1 plasmid DNA. *Proc. Natl. Acad. Sci. USA* **74** (5), 1865–1869.

Torreblanca, M., Meseguer, I., and Rodriguez-Valera, F. (1989). Halocin H6, a bacteriocin from *Haloferax gibbonsii*. *J. Gen. Microbiol.* **135,** 2655–2661.

Wu, S., Dornbusch, K., Kronvall, G., and Norgren, M. (1999). Characterization and nucleotide sequence of a *Klebsiella oxytoca* cryptic plasmid encoding a CMY-type B-lactamase: Confirmation that the plasmid-mediated cephamycinase originated from the *Citrobacter freundii* amp C B-lactamase. *Antimicrob. Agents Chemother.* **43,** (6), 1350–1357.

Zverev, V. V., and Khmel, I. A. (1985). The nucleotide sequences of the replication origins of plasmids ColA and ColD. *Plasmid* **14,** 192–199.

Communicated by David H. Figurski