JOURNAL OF BACTERIOLOGY, Apr. 1996, p. 2431–2435 Vol. 178, No. 8
0021-9193/96/$04.00+0

Periplasmic Location of the Pesticin Immunity Protein Suggests Inactivation of Pesticin in the Periplasm

HOLGER PILSL, HELMUT KILLMANN, KLAUS HANTKE, AND VOLKMAR BRAUN*

Mikrobiologie II, Universität Tübingen, D-72076 Tübingen, Germany

Received 8 November 1995/Accepted 8 February 1996

The pesticin activity and immunity genes on plasmid pPCP1 of *Yersinia pestis* were sequenced. They encoded proteins of 40 kDa (pesticin) and 16 kDa (immunity protein); the latter was found in the periplasm. The location of the immunity protein suggests that imported pesticin is inactivated in the periplasm before it hydrolyzes murein. Pesticin contains a TonB box close to the N-terminal end that is identical to the TonB box of colicin B. The DNA sequences flanking the pesticin determinant were highly homologous to those flanking the colicin 10 determinant. It is proposed that through these highly homologous DNA sequences, genes encoding bacteriocins may be exchanged between plasmids by recombination. In the case of pesticin, recombination may have destroyed the lysis gene, of which only a rudimentary fragment exists on pPCP1.

Bacteriocins are protein toxins synthesized by bacteria that kill only bacteria that are closely related to the producing species; e.g., colicins produced by *Escherichia coli* kill only *E. coli* and closely related members of the family *Enterobacteriaceae* (3). The narrow host range is determined by a highly specific uptake of the proteins into sensitive cells, which in gram-negative bacteria requires an outer membrane receptor protein and in the case of the Ton-dependent colicins input of energy provided by the electrochemical potential of the cytoplasmic membrane. In *E. coli*, energy flux from the cytoplasmic membrane into the outer membrane is mediated by a protein complex consisting of the proteins TonB, ExbB, and ExbD (Ton system) (2, 19, 26). Ton-dependent receptors and colicins contain a common signature close to the N-terminal end that consists of a homologous pentapeptide, the so-called TonB box. Mutations in the TonB box of receptors (1, 15, 33) and colicins (20, 25) impair uptake, but not binding of colicins to the receptors, and they are suppressed by mutations in TonB. Release of colicins by the producing cells is much less specific than colicin uptake. It occurs by quasilysis mediated by a lysis protein that disrupts the permeability barrier of the cytoplasmic membrane and activates in *E. coli* an outer membrane phospholipase (6, 27). Some colicinogenic determinants lack a lysis gene; therefore, the bulk of these colicins stays within the cells. Colicin-producing cells are protected by immunity proteins that usually interact very selectively with the related colicin (18). Colicins are always encoded on plasmids, and the activity (*a*), immunity (*i*), and lysis (*l*) genes map close to each other in the order *a*, *i*, *l*.

The majority of colicins act by forming pores in the cytoplasmic membrane that disrupt the transmembrane potential or by hydrolyzing DNA or 16S rRNA (3, 6, 18). Among the few bacteriocins of gram-negative bacteria that do not form pores or act as nucleases are colicin M, which interferes with murein and O-antigen biosynthesis by inhibiting the regeneration of the common lipid carrier (13, 14), and pesticin of *Yersinia pestis*, which hydrolyzes murein (9). Both bacteriocins convert cells to spheroplasts (4, 12). For uptake into sensitive cells, pesticin uses the FyuA receptor (28, 29), which normally translocates a ferric siderophore, yersiniabactin (7), through the outer membrane. Many colicins, e.g., colicins B, D, M, Ia, Ib, and V (3) (the latter is, in fact, a microcin [11]), use ferric siderophore receptors for binding to the outer membrane. In addition, pesticin uptake is *tonB* dependent (10), as is the uptake of the above-listed colicins. After SOS induction of pesticin-producing cells, only low amounts of pesticin are found in the spent medium, and most of the activity remains cell associated (17), suggesting the lack of a functional lysis protein. Since pesticin has only to enter the periplasmic space where murein resides, the immunity protein should be located in this compartment. It has been shown previously that pesticin purified from a crude cell extract consisted of a single polypeptide (16), although the molecular weight of the native protein was determined to be 66,000 by ultracentrifugation, 63,000 by gel filtration in 6 M guanidine chloride, and 44,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16). However, no additional protein bands were observed after SDS-PAGE, and the purified protein was active in vitro; therefore, it appears that the immunity protein is not associated with pesticin, in contrast to the colicins displaying nuclease activities, which contain tightly bound immunity proteins in the intracellular form as well as in the released form (18). The pore-forming colicins do not contain bound immunity proteins, and it is thought that they are inactivated when they enter the cytoplasmic membrane from outside the cells (8, 23, 40).

Since the intracellular location of pesticin inactivation by its cognate immunity protein may differ from that of any other gram-negative bacteriocin described, we studied the location of the pesticin immunity protein in the bacterial cell. We were also interested in the primary structure of pesticin because of its unusual activity and, in its true molecular weight, the presence of a TonB box and possible homologies to colicins. The latter are composed of domains that determine receptor specificity, uptake across the outer membrane via the Ton or the Tol system (3, 39), activity, and binding of the immunity protein. Since nearly identical domains can be found in otherwise different colicins, we have proposed that during evolution, colicins were assembled by combining DNA fragments that encode functional domains (22, 24, 30).

Sequencing of the pesticin activity and immunity genes. The pesticin determinant is encoded by a 6-kb *Bgl*II fragment of the natural 9.5-kb plasmid pPCP1 (Fig. 1) (35). A 2,007-bp *Mae*II-*Xho*II fragment of the isolated *Bgl*II fragment was completely

* Corresponding author. Mailing address: Mikrobiologie II, Auf der Morgenstelle 28, D-72076 Tübingen, Germany. Phone: 49 7071 292096. Fax: 49 7071 294634.

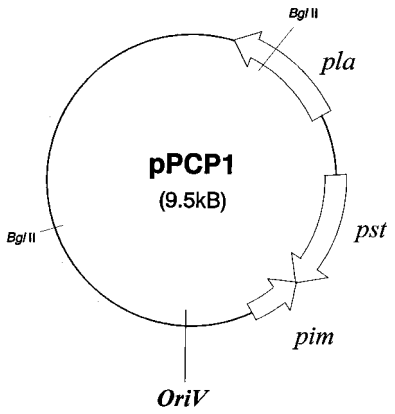


FIG. 1. Arrangement of the *pst*, *pim*, and *pla* genes on the natural plasmid pPCP1 (35). The arrows indicate transcriptional polarity. The *Bgl*II restriction sites were used for cloning of the pesticin genes.

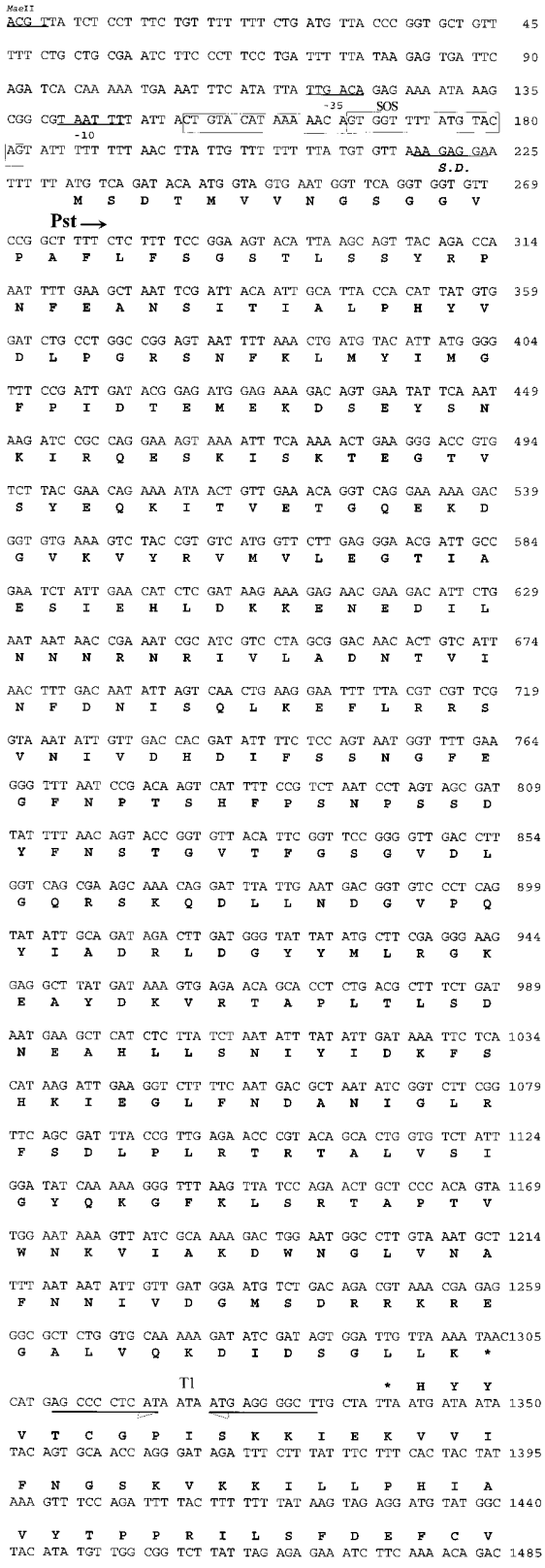
sequenced in both directions by the dideoxy-chain termination method (32). It contained two open reading frames with opposite transcription polarities (Fig. 2), which were named *pst* and *pim* in accordance with a previous proposal (35). The region between *pst* and *pim* could form a hairpin loop that probably serves as a transcription termination site for both genes (T1 in Fig. 2). *pst* encodes a protein composed of 357 residues (M_r, 40,043), and *pim* encodes a protein of 141 residues (M_r, 16,022). SDS-PAGE of pesticin purified by chromatography on a Resource Q cation-exchange column (Pharmacia Biotec, Uppsala, Sweden) revealed a single protein with an electrophoretic mobility corresponding to an M_r of 42,000 (data not shown). No other protein was seen on the gel, showing that pesticin is produced by the cells without a tightly associated immunity protein.

Comparison of the pesticin sequence with those of other colicins revealed a TonB box (DTMVV, residues 3 to 7) that is identical to the TonB box of colicin B (34). Pesticin was recognized by the *E. coli* Ton system since *E. coli* K-12 transformed with *fyuA*, encoding the pesticin receptor FyuA, and certain *E. coli* isolates that produce the pesticin receptor (10, 28) were sensitive to pesticin. No other sequence homologies were found, as expected in view of the unique activity of pesticin compared with other characterized bacteriocins. However, the nucleotide sequence upstream of the *pst* coding region displayed 75.3% identity with the region upstream of the colicin 10 open reading frame (Fig. 3A) (22). A sequence upstream of *pim* exhibited 93.9% identity with the sequence within and downstream of the colicin 10 lysis gene (Fig. 3B) (22). The conserved DNA sequences upstream and downstream of the pesticin determinant might have served as recombination sites for the insertion and exchange of bacteriocin determinants on plasmids. The pesticin genes on pPCP1 may have replaced a colicin 10-like determinant in such a way that only a nonfunctional lysis gene (*psl**) has remained. The putative transcription termination structure (T2 in Fig. 3B) downstream of *psl** is also present downstream of *ctl* on pCol10 (22), so that T2, like *psl**, may be an evolutionary relic. Exchange of colicin determinants among plasmids is further supported by the strong homologies of the flanking regions of the pesticin and colicin 10 determinants to the DNA regions flanking the determinants of colicins 5, K, and E1 (22–24). Plasmids pCol10, pCol5, pColK, and pColE1 are similar in size (5.2 to 7 kb) and structure. Plasmid pPCP1 is somewhat larger (9.5 kb) and encodes an additional gene, the plasminogen activator gene *pla* (Fig. 1) (35). Further evidence that the plasmids pPCP1 and pColE1 are evolutionary closely related comes from their *polA*-dependent replication and their incompatibility (35). A high degree of sequence identity (85% over a stretch of 294 nucleotides) was also found upstream of the activity genes of colicins B and E1 (34), although both plasmids and colicins otherwise differ entirely.

Subcellular localization of the pesticin immunity protein. The N-terminal end of the Pim immunity protein displays the characteristics of a signal sequence. It is composed of a positively charged N-terminal end, followed by a stretch of hydrophobic amino acids (residues 5 to 19) which at the end contain the potential signal peptidase I cleavage site GLA. The rest of the molecule is hydrophilic. Therefore, we assumed that the N terminus translocates Pim into the periplasm and either is cleaved or anchors Pim to the cytoplasmic membrane. To determine the location of Pim, we used the β-lactamase fusion technique (5). *E. coli* 5K was made pesticin sensitive by transformation with plasmid pHM10, which encodes the FyuA pesticin receptor (28). Blunt-end restriction sites were introduced by PCR at various locations in the *pim* gene and used to fuse *pim* and 3′ truncated *pim* fragments to a 5′ truncated *blaM* gene that did not encode the signal sequence. ′BlaM was encoded by plasmid pJBS636, a derivative of pJBS633 (5) into which the phage T7 promoter DNA had been inserted (23). Only those hybrid proteins in which the β-lactamase moiety was translocated by Pim and its derivatives across the cytoplasmic membrane into the periplasm conferred resistance to ampicillin. The resulting hybrid proteins contained 20, 40, 60, 89, 108, and 141 residues of Pim and the mature β-lactamase (Table 1). The correctness of the hybrid genes was verified by DNA sequencing.

For identification of the hybrid proteins, *E. coli* BL21 (37) was transformed with the plasmids that contained the *pim′-′blaM* genes downstream of the gene 10 promoter of phage T7. The genes were transcribed by T7 RNA polymerase, and the proteins were labeled with [^{35}S]methionine (38). The autoradiographs of the labeled proteins separated by SDS-PAGE revealed radioactive bands of the expected sizes (Fig. 4, lanes 4 to 9; unprocessed hybrid proteins are marked by arrows). The proteins were strongly degraded to defined products that were all larger than processed β-lactamase, indicating that proteolytic cleavage occurred mainly in the immunity protein. It is not clear whether the immunity protein portion was completely degraded, since smaller degradation products may have escaped detection because of the weak labeling of Pim, which contains only one methionine residue (or two if the N-terminal methionine is not cleaved). β-Lactamase of the hybrid proteins was probably as stable as the free form (Fig. 4, lane 3), since no smaller products of the strongly labeled protein were observed. Wild-type Pim was also proteolytically degraded (Fig. 4, lane 1). Because of the low methionine content of Pim, the gel representing lane 1 had to be exposed to the X-ray film three times longer than the gel of lanes 2 to 9.

All of the hybrid proteins conferred ampicillin resistance (Table 1) at a level far above the background resistance of the untransformed strain (below 5 μg/ml). The two shortest hybrid proteins caused the highest resistance. To examine whether the proteolytic degradation reduced the level of ampicillin resistance and to identify the protease that degraded the hybrid proteins, the plasmids were transformed into *E. coli* KS474 *degP* lacking a periplasmic protease (36) and into *E. coli* UT5600 *ompT* lacking an outer membrane protease (31). Five of the hybrids conferred a higher level of ampicillin resistance in the *ompT* mutant than in *E. coli* 5K cells (Table 1). The Pim-Bla hybrid proteins of KS474 *degP* and UT5600 *ompT*



cells were degraded to products similar to those of strain BL21 (data not shown); therefore, OmpT and presumably DegP are not the only proteases that contribute to Pim degradation. Proteolytic degradation may be caused by overproduction of

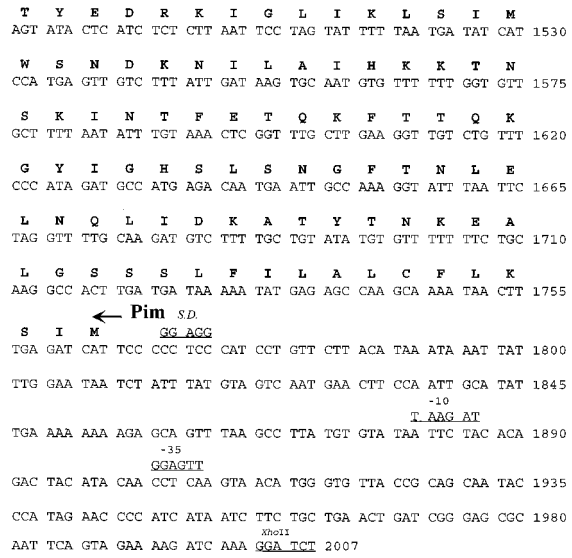


FIG. 2. Nucleotide sequences of *pst* and *pim*. The arrows indicate transcriptional polarity. SOS, SOS boxes (boxed); S.D., Shine-Dalgarno sequences (underlined); −10 and −35, putative promoter region; T1, predicted transcription termination site.

the proteins and may not affect immunity displayed by Pim expressed by pPCP1. The data indicate that all of the hybrid proteins contain the β-lactamase in the periplasm, demonstrating location of the Pim-BlaM fusion sites in the periplasm.

The strong proteolysis of the Pim-BlaM hybrid proteins could also not be prevented by adding various inhibitors of proteases during the preparation of cells and cell fractions. Therefore, we added sodium azide for 20 min prior to radioactive labeling of the proteins to examine whether inhibition of protein export across the cytoplasmic membrane resulted in the accumulation of the precursors of Pim and the Pim-BlaM hybrid proteins that still contained the signal sequence. We obtained for some of the hybrid proteins a band above the mature form suggesting inhibition of processing (data not shown). Also in these experiments, proteolysis prevented an unequivocal interpretation. However, the electrophoretic position of Pim20-BlaM (Fig. 4, lane 4) corresponded to the position of processed BlaM (Fig. 4, lane 3), indicating that the Pim20 fragment was cleaved from BlaM. It is more likely that the Pim20 fragment was cleaved by the signal peptidase 1 than by unspecific proteases, since the Pim20 fragment is probably protected by the membrane.

We also determined whether the Pim-Bla hybrid proteins conferred immunity to added pesticin. *E. coli* 5K(pHM10 *fyuA*) was sensitive to a 10^3-fold diluted pesticin sample and was fully immune to undiluted pesticin after transformation with pHP70 *pim*. Of the Pim-Bla hybrids, only Pim141-Bla, containing a full-size Pim, conferred partial immunity in that cells were immune to 10^3- and 10^2-fold-diluted pesticin samples but were sensitive to 10-fold-diluted pesticin.

The only colicin immunity protein which up to now has been localized in the periplasm is that of colicin M (11a, 21). Colicin M and pesticin are the only known bacteriocins that damage the murein, by inhibition of murein biosynthesis and by murein hydrolysis. The target of colicin M is in the cytoplasmic membrane, and it makes sense that the colicin M immunity protein is anchored to the cytoplasmic membrane by its N-terminal hydrophobic end (11a). The colicin M immunity protein may inactivate colicin M during its import, and the pesticin immunity protein may prevent hydrolysis of murein by Pst.

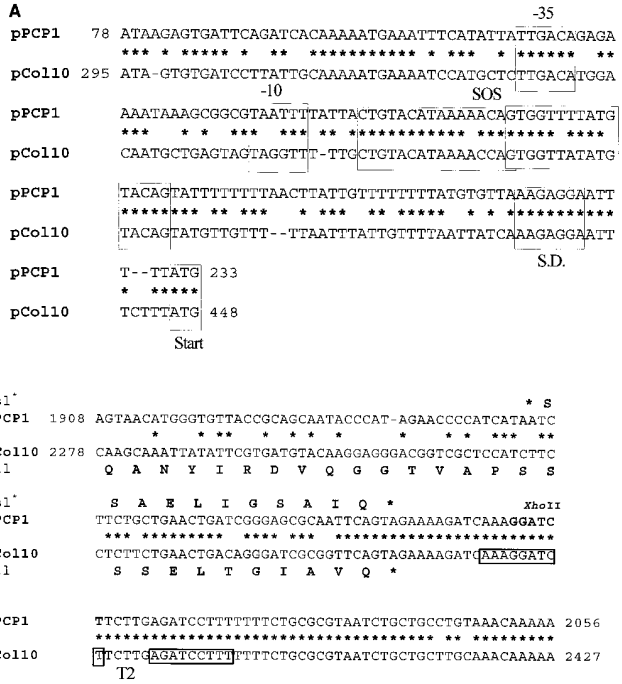


FIG. 3. (A) Sequence similarity between the upstream region of *pst* (bp 78 to 233) on pPCP1 and the upstream region of *cta* (bp 295 to 448) on pCol10 (22). The −10 and −35 promoter regions, the SOS boxes, the Shine-Dalgarno sequences (S.D.), and the start codons are indicated (boxed). Asterisks denote identical bases. (B) The sequence similarity between the upstream region of *pim* on pPCP1 (bp 1908 to 2056) and the region upstream of the colicin 10 immunity gene (*cti*) on pCol10 (bp 2278 to 2427) starts inside the colicin 10 lysis gene (*ctl*). The deduced amino acid sequences of Ctl and of the rudimentary lysis protein Psl* are shown. T2 is a potential transcription terminator for the colicin 10 genes (22).

After our sequencing of the *pst* and *pim* genes had been completed, the sequence of the pesticin structural gene was deposited by two groups in the EMBL sequence data library. The *pst* sequence of the plasmid used in this study (accession number U31974) agrees completely with the *pst* sequence determined in our study; the sequence determined for a distinct plasmid (pYP358; accession number Z54145) differs at nucleotide number 927 in Fig. 2 (A instead of T, Asn instead of Tyr).

Nucleotide sequence accession number. The sequence shown in Fig. 2 has been deposited in the EMBL nucleotide sequence data library (accession number X92856).

TABLE 1. Ampicillin resistance of *E. coli* strains expressing Pim–β-lactamase chimeras[a]

Plasmid	Ampicillin (μg/ml)		
	5K	KS474 *degP*	UT5600 *ompT*
None	<5	<5	<5
pHP71 (Pim20-Bla)	800	800	1,600
pHP72 (Pim40-Bla)	800	800	1,600
pHP73 (Pim60-Bla)	200	200	200
pHP74 (Pim89-Bla)	50	50	200
pHP75 (Pim108-Bla)	100	100	400
pHP76 (Pim141-Bla)	100	200	200

[a] Resistance to ampicillin was tested on nutrient agar plates that contained the indicated ampicillin concentrations. *E. coli* 5K, KS474 *degP*, and UT5600 *ompT* were transformed with the listed plasmids. Ampicillin resistance above 5 μg/ml indicate a Pim′-′BlaM fusion site located in the periplasm (5).

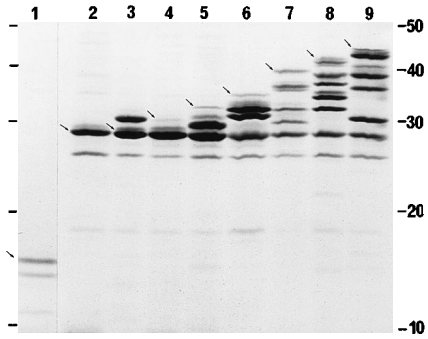


FIG. 4. SDS-PAGE of [^{35}S]methionine-labeled Pim (lane 1), Pim20-Bla (lane 4), Pim40-Bla (lane 5), Pim60-Bla (lane 6), Pim89-Bla (lane 7), Pim108-Bla (lane 8), and Pim141-Bla (lane 9) expressed in *E. coli* BL21 (unprocessed proteins are indicated by arrows) with plasmids pHP69 and pHP71 to pHP76, respectively. Lane 3 shows the precursor and the processed form of β-lactamase (indicated by an arrow) expressed by plasmid pT7-3, and lane 2 shows the neomycin phosphotransferase expressed by the vector pJBS636. Sizes are indicated in kilodaltons.

We thank A. Rakin for providing plasmid pHM10 and Karen A. Brune for reading the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 323, project B1, and Graduiertenkolleg Mikrobiologie [H.P.]).

REFERENCES

1. **Bell, P. E., C. D. Nau, J. T. Brown, J. Konisky, and R. J. Kadner.** 1990. Genetic suppression demonstrates interaction of TonB protein with outer membrane transport proteins in *Escherichia coli*. J. Bacteriol. **175:**3146–3150.
2. **Braun, V.** 1995. Energy-coupled transport and signal transduction through the gram-negative outer membrane via TonB-ExbB-ExbD-dependent receptor proteins. FEMS Microbiol. Rev. **16:**295–307.
3. **Braun, V., H. Pilsl, and P. Groß.** 1994. Colicins: structures, modes of action, transfer through membranes, and evolution. Arch. Microbiol. **161:**199–206.
4. **Braun, V., K. Schaller, and R. W. Wabl.** 1974. Isolation, characterization, and action of colicin M. Antimicrob. Agents Chemother. **5:**520–533.
5. **Broome-Smith, J. K., and B. G. Spratt.** 1986. A vector for the construction of translational fusions to TEM β-lactamase and the analysis of protein export signals and membrane protein topology. Gene **49:**341–349.
6. **De Graaf, F. K., and B. Oudega.** 1986. Production and release of cloacin DF13 and related colicins. Curr. Top. Microbiol. Immunol. **125:**183–205.
7. **Drechsel, H., H. Stephan, R. Lotz, H. Haag, H. Zähner, K. Hantke, and G. Jung.** 1995. Structure elucidation of Yersiniabactin, a siderophore from highly virulent *Yersinia* strains. Liebigs Ann. Chem. **1995:**1727–1733.
8. **Espesset, D., P. Piet, C. Lazdunski, and V. Geli.** 1994. Immunity proteins to pore-forming colicins: structure-function relationships. Mol. Microbiol. **13:** 1111–1120.
9. **Ferber, D. M., and R. R. Brubaker.** 1979. Mode of action of pesticin: *N*-acetylglucosaminidase activity. J. Bacteriol. **139:**495–501.
10. **Ferber, D. M., J. M. Fowler, and R. R. Brubaker.** 1981. Mutations to tolerance and resistance to pesticin and colicins in *Escherichia coli* Φ. J. Bacteriol. **146:**506–511.
11. **Gilson, L., H. K. Mahanty, and R. Kolter.** 1990. Genetic analysis of an MDR-like export system: the secretion of colicin V. EMBO J. **9:**3875–3884.

11a. **Groß, P., and V. Braun.** Colicin M is inactivated during import by its immunity protein. Mol. Gen. Genet., in press.

12. **Hall, P. J., and R. R. Brubaker.** 1978. Pesticin-dependent generation of osmotically stable spheroplast-like structures. J. Bacteriol. **136:**786–789.
13. **Harkness, R. E., and V. Braun.** 1989. Colicin M inhibits peptidoglycan biosynthesis by interfering with lipid carrier recycling. J. Biol. Chem. **264:** 6177–6182.
14. **Harkness, R. E., and V. Braun.** 1989. Inhibition of lipopolysaccharide O-antigen synthesis by colicin M. J. Biol. Chem. **264:**14716–14722.
15. **Heller, K. J., R. J. Kadner, and K. Günther.** 1988. Suppression of the *btuB*451 mutation by mutations in the *tonB* gene suggests a direct interaction between TonB and TonB-dependent receptor proteins in the outer membrane of *Escherichia coli*. Gene **64:**147–153.
16. **Hu, P. C., and R. R. Brubaker.** 1974. Characterization of pesticin: separation of antibacterial activities. J. Biol. Chem. **249:**4749–4753.
17. **Hu, P. C., G. C. H. Yang, and R. R. Brubaker.** 1972. Specificity, induction, and absorption of pesticin. J. Bacteriol. **112:**212–219.

18. **James, R., C. Lazdunski, and F. Pattus.** 1992. Bacteriocins, microcins and lantibiotics. NATO ASI Series H. Cell biology, vol. 65. Springer Verlag, Berlin.
19. **Kadner, R. J.** 1990. Vitamin B_{12} transport in *Escherichia coli*: energy coupling between membranes. Mol. Microbiol. **4:**2027–2033.
20. **Mende, J., and V. Braun.** 1990. Import-defective colicin B derivatives mutated in the TonB box. Mol. Microbiol. **4:**1523–1533.
21. **Ölschläger, T., A. Turba, and V. Braun.** 1991. Binding of the immunity protein inactivates colicin M. Mol. Microbiol. **5:**1105–1111.
22. **Pilsl, H., and V. Braun.** 1995. Novel colicin 10: assignment of four domains to the TonB- TolC-dependent uptake via the Tsx receptor and to pore formation. Mol. Microbiol. **16:**57–67.
23. **Pilsl, H., and V. Braun.** 1995. Evidence that the immunity protein inactivates colicin 5 immediately prior to the formation of the transmembrane channel. J. Bacteriol. **177:**6966–6972.
24. **Pilsl, H., and V. Braun.** 1995. Strong function-related homology between the pore-forming colicins K and 5. J. Bacteriol. **177:**6973–6977.
25. **Pilsl, H., C. Glaser, P. Groß, H. Killmann, T. Ölschläger, and V. Braun.** 1993. Domains of colicin M involved in uptake and activity. Mol. Gen. Genet. **240:**103–112.
26. **Postle, K.** 1993. TonB protein and energy transduction between membranes. J. Bioenerg. Biomembr. **25:**591–601.
27. **Pugsley, A. P., and M. Schwartz.** 1984. Colicin E2 release: lysis, leakage or secretion? Possible role of a phospholipase. EMBO J. **3:**2393–2397.
28. **Rakin, A., E. Saken, D. Harmsen, and J. Heesemann.** 1994. The pesticin receptor of *Yersinia enterocolitica*: a novel virulence factor with dual function. Mol. Microbiol. **13:**253–263.
29. **Rakin, A., P. Urbitsch, and J. Heesemann.** 1995. Evidence for two evolutionary lineages of highly pathogenic *Yersinia* species. J. Bacteriol. **177:**2292–2298.
30. **Roos, U., R. E. Harkness, and V. Braun.** 1989. Assembly of colicin genes from a few DNA fragments. Nucleotide sequence of colicin D. Mol. Microbiol. **3:**891–902.
31. **Rupprecht, K. R., G. Gordon, M. Lundrigan, R. C. Gayda, A. Markovitz, and C. Earhart.** 1982. *ompT*: *Escherichia coli* K-12 structural gene for protein a (3b). J. Bacteriol. **153:**1104–1106.
32. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
33. **Schöffler, H., and V. Braun.** 1989. Transport across the outer membrane of *Escherichia coli* K12 via the FhuA receptor is regulated by the TonB protein of the cytoplasmic membrane. Mol. Gen. Genet. **217:**378–383.
34. **Schramm, E., J. Mende, V. Braun, and R. M. Kamp.** 1987. Nucleotide sequence of colicin B activity gene *cba*: consensus pentapeptide among TonB-dependent colicins and receptors. J. Bacteriol. **169:**3350–3357.
35. **Sodeinde, O. A., and J. D. Goguen.** 1988. Genetic analysis of the 9.5-kilobase virulence plasmid of *Yersinia pestis*. Infect. Immun. **56:**2743–2748.
36. **Strauch, K. L., K. Johnson, and J. Beckwith.** 1989. Characterization of *degP*, a gene required for proteolysis in the cell envelope and essential for growth of *Escherichia coli* at high temperature. J. Bacteriol. **171:**2689–2696.
37. **Studier, F. W., and B. A. Moffat.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189:**113–130.
38. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
39. **Webster, R. E.** 1991. The *tol* gene products and the import of macromolecules into *Escherichia coli*. Mol. Microbiol. **5:**1005–1011.
40. **Zhang, Y. L., and W. A. Cramer.** 1993. Intramembrane helix-helix interaction as the basis of inhibition of the colicin E1 ion channel by its immunity protein. J. Biol. Chem. **268:**10176–10184.