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ATP Synthase Is Necessary for Microcin H47 Antibiotic Action

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Microcin H47 is a gene-encoded peptide antibiotic produced by a natural *Escherichia coli* strain isolated in Uruguay. In order to identify cellular components necessary for its antibiotic action, microcin H47-resistant mutants isolated in this work, as well as previously described mutants affected in membrane proteins, were analyzed. These studies indicated that (i) receptor outer membrane proteins for ferric-catechol siderophores would be involved in microcin-specific binding to the cell surface, (ii) the TonB pathway is needed for microcin H47 uptake, and (iii) the presence of the ATP synthase complex is necessary for microcin action. The possibility that this last structure contains the antibiotic target is discussed.

Microcins are a group of antibiotic peptides produced by strains of gram-negative bacteria. They are synthesized as gene-encoded precursors that thereafter undergo posttranslation modifications, thus converting to mature molecules. In general terms, they are considered to be narrow-spectrum antibiotics that act against gram-negative bacteria, some microcins being bactericidal while others are bacteriostatic (3, 17, 19).

Although at present only a few microcins have been thoroughly studied, it is already clear that they exhibit a noteworthy diversity in their mechanism of action. To exert their deleterious effect on sensitive cells, they have to transit two steps: microcin uptake and interaction with their targets. In some cases, uptake has been described as starting by binding of microcin to a receptor protein in the cell surface; the following outer membrane proteins were described as being involved in such interactions: Cir for colicin V, the porin OmpF for microcin B17, and FhuA for microcin J25 (8, 19, 20, 34). To pass through the outer membrane barrier, the TonB pathway is used by several microcins, e.g., E492, J25, and colicin V (28, 35). Finally, microcins B17 and J25 need the presence of a cytoplasmic membrane protein, SbmA, to complete their uptake (20, 35).

It is the final interaction with their targets that points to microcins as antibiotics with interesting modes of action: colicin V and microcin E492 disrupt membrane potential (9, 42), microcin B17 inhibits DNA replication by irreversibly trapping DNA gyrase-DNA complexes (38), microcin C7 inhibits protein synthesis (13), and microcin J25 was proposed to interfere with cell division (33).

Here we present a study on the mode of action of microcin H47 (MccH47). MccH47, which was found to be produced by a natural *Escherichia coli* isolate, inhibits the growth of gram-negative strains. The MccH47 genetic system was located in the chromosome, with an extension of ca. 10 kb (21). In brief, it comprises four genes involved in microcin synthesis: the microcin structural gene, two further genes devoted to microcin secretion, and an immunity gene whose product protects the cell against its own antibiotic production (2, 12, 30, 31). MccH47 is ribosomally synthesized as a peptide precursor which already possesses antibiotic activity, detected as a toxic effect in cells carrying the microcin structural gene *mchB* and lacking the remaining *mch* determinants. Although cells with such a genetic construction were nonviable, some mutant clones grew which exhibited Atp^- and MccH47-resistant ($MccH47^r$) phenotypes (31). These results suggested that ATP synthase could be involved in microcin action on susceptible cells. The present work deals with the MccH47 mode of action and presents results that point to ATP synthase as the possible target of this antibiotic.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. The strains, plasmids, and bacteriophages used in this study are listed in Table 1.

Media and culture conditions. Luria-Bertani (LB)-rich medium, R medium for phage lysates, and M63 minimal medium supplemented either with glucose or succinate were used (24). Antibiotics were added to media at the following final concentrations: ampicillin (Ap), 50 μg/ml; chloramphenicol (Cm), 30 μg/ml; kanamycin (Km), 30 μg/ml; and tetracycline (Tc), 12 μg/ml. To prepare iron-chelated plates, ethylenediamine DI(*o*-hydroxyphenylacetic acid) (EDDHA) was added at a final concentration of 50 μM. All strains were grown at 37°C, except for those carrying Mu*cts*, which were grown at 30°C.

M63 glucose plates containing MccH47 were prepared as follows: the M63 plates were covered with a cellophane membrane, and about 10^7 *E. coli* RYC1000(pEX100) cells, which overproduce MccH47, were poured above with soft agar. After 24 h of incubation, the cellophane with the producing lawn was removed and the plates were exposed to chloroform vapors to kill cells that could have remained. These plates contained MccH47 that had passed through the cellophane membrane. These MccH47-containing plates were then used for selecting MccH47-resistant clones.

Microcin, colicin, and bacteriophage ϕ80 sensitivity assays. The cross-streaking method was employed on LB plates: a suspension of the microcin- or colicin-producing strain was seeded as a band and incubated during 24 h; the clones to be assayed were then cross-streaked, and the plates were further incubated. Sensitive clones were unable to grow in the vicinity of the producing band, while growth of resistant clones was not inhibited. The sensitivity to phage ϕ80 was also assayed by cross-streaking. Mutants' sensitivity to MccH47 was rapidly screened by the cross-streaking method, and thereafter, selected mutants were analyzed with more precision by a patch test (29). This test was performed by seeding a

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TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain, phage, or plasmid	Genotype	Source or reference
E. coli K-12 strains		
BZB1011	*gyrA*	Laboratory collection
FGB11	BZB1011 *tonB*::Tn*5*	This work
FGB46	BZB1011 *exb* Tn*5*	This work
FGB64	BZB1011 *atpB*::Tn*5 64*	This work
KS463	*rha-7 IN(rrnD-rrnE)1 trpA33*	B. Konrad (CGSC 5644)
MC4100	F$^-$ *araD139 lacU169 relA rpsL thiA*	Laboratory collection
H1728	MC4100 *aroB cir fiu*::Mu*d*1X	15
H1876	H1728 *fepA*::Tn*10*	15
RYC1000	F$^-$ *araD139 gyrA lacU169 rbs7 relA rpsL thiA*	12
Bacteriophages		
λ-467	*b221 cI857 Oam29 Pam80 rex*::Tn5 (Km^r)	4
Mu*cts* Ap^r	Mu*cts* carrying the *bla* gene of Tn*3*	22
Plasmids		
pEX100	pACYC184 carrying the MccH47 system (Cm^r)	12
pMVD10	pACYC184 carrying *mchB72*::*phoA* (Cm^r)	31
pEG109	Mu*d*II4042::*phoA proC* (Cm^r)	14
pCH9	pWSK29 with *exbBD* (Ap^r)	7
pIM91	pSU18 with *tonB* (Cm^r)	18
pBJC917	pBR322 carrying *atpBEFHAGDC* (Ap^r)	39

lawn of each strain to be assayed on minimal glucose plates and then stabbing the lawn with a MccH47-overproducing strain, RYC1000(pEX100). After incubation, halos of growth inhibition appeared when the strain assayed was sensitive to MccH47, the level of sensitivity being estimated by the diameter of the zone of inhibition.

To quantify sensitivity of BZB1011 to MccH47 under low-iron conditions, the critical dilution method was employed (23) using MccH47 extracts prepared as described previously (12). Briefly, 10 μl of twofold dilutions of a MccH47 extract was spotted onto a lawn of BZB1011 in LB plates, with and without the addition of the iron chelator EDDHA. After incubation, growth inhibition halos appeared. The highest dilution giving rise to a clear halo was taken as an arbitrary measure of sensitivity to MccH47.

Genetic techniques. P1 transduction was performed as described previously (24). Six independent Tn*5* mutagenesis experiments were carried out by infecting BZB1011 with phage λ 467 as described previously (4). MccH47^r Tn*5* insertion mutants were selected on M63 glucose MccH47 Km plates.

Mu*d*II4042 in vivo cloning. The Tn*5* insertion in FGB64 was cloned in vivo from the chromosome by means of lytic propagation of phage Mu*d*II4042 in FGB64 as described previously (14). For this purpose, FGB64 was first lysogenized with Mu*cts*Ap^r and then transformed with the Mu*d*II4042-containing plasmid pEG109, and the resultant strain was induced for phage propagation. The transducing phage lysate was used to infect MC4100 Mu*cts*Ap^r, and transductant clones were selected on LB plates containing Cm and Km.

Manipulation and sequencing of DNA. Routine DNA manipulations and plasmid vectors used were described previously (36). DNA sequence was elucidated by applying the chain termination method to double-stranded DNA using the Sequenase 2.0 kit from United States Biochemicals (Cleveland, Ohio) (37). Universal and reverse sequencing primers were used.

RESULTS AND DISCUSSION

Isolation and preliminary characterization of MccH47-resistant mutants. Strain BZB1011 was mutagenized with transposon Tn*5* as described in Materials and Methods. Km^r MccH47^r mutants appeared at a frequency of 10^{-5} of the viable clones. Most mutants exhibited normal growth, similar to that of BZB1011, while others grew poorly in LB and M63 glucose media. Their level of MccH47 resistance was evaluated by a patch test as described in Materials and Methods, finding two phenotypes: partially resistant mutants and highly resistant mutants. The diameter of the growth inhibition halos of the partially resistant mutants presented a mean value of 6 mm, whereas no inhibition was observed with the highly resistant mutants. In the same assays, the diameter of inhibition of BZB1011 was 19 mm, on average.

Taking into account that cross-resistance analyses could give information on the nature of mutations conferring resistance to MccH47, Tn*5* mutants were tested for their sensitivities to several colicins and microcins. Three different phenotypes were found: sensitivity to all tested colicins and microcins, resistance to colicins M and V, and resistance to colicin M only.

Based on their phenotype, MccH47^r mutants were preliminarily classified into three groups: (i) mutants with normal growth, highly resistant to MccH47 and also resistant to colicins M and V; (ii) mutants with normal growth, partially resistant to MccH47 and resistant to colicin M; and (iii) mutants exhibiting poor growth, completely resistant to MccH47 and sensitive to all tested colicins and microcins.

***tonB* mutants.** Clones from the first group were resistant to colicins M and V, which have in common the use of the TonB pathway for their uptake (28). Since bacteriophage ϕ80 is known to require the presence of TonB to infect cells (27), 16 mutants were analyzed and were found to be insensitive to this phage. The *tonB* gene maps to minute 28 of the *E. coli* K12 chromosomal map, near the *trp* locus. The P1 transducing phage was propagated on four MccH47^r independent mutants from this group, and the lysates were used to infect the *trpA* MccH47^s strain KS463, selecting prototrophic transductant clones on minimal M63 glucose plates. Transductants from each experiment were assayed for their sensitivity to MccH47, and 38 to 67% were resistant to this antibiotic. When tested for their sensitivity to kanamycin, a strict correlation was found between the MccH47^r and Km^r phenotypes. These results indicated that mutations conferring microcin resistance mapped near *trpA* and that they had occurred by Tn*5* insertion. Finally, the plasmid pIM91, carrying only the *tonB* gene, restored microcin H47 sensitivity when introduced into the same four mutant strains. One of these strains was selected as the prototype of this group and was called FGB11. In sum, mutants

from the first group were shown to be TonB deficient, indicating that MccH47 makes use of the TonB pathway to cross the outer membrane of sensitive cells.

***exb* mutants.** Mutants classified in the second group were resistant to colicin M and partially resistant to MccH47. They also were sensitive to bacteriophage ϕ80. This phenotype could correspond to mutants affected in *fhuA*, which encodes the outer membrane receptor of colicin M and of the iron carrier ferrichrome (8, 16), or in *exb*, a locus comprising two genes, *exbB* and *exbD*, whose products accomplish ancillary functions in the process of active transport through the outer membrane mediated by TonB (1). A well-known *fhuA* deletion mutant, H1857 (6), proved to be sensitive to MccH47, thus ruling out FhuA involvement in MccH47 uptake. On the contrary, the *exb* strain W3110-6 (11) presented a partial level of resistance to this antibiotic, similar to that of MccH47^r mutants under study. The plasmid pCH9, carrying only the *exbBD* genes, was introduced into four MccH47^r mutants from the second group, and the transformant clones that resulted were sensitive to MccH47. One of these mutants was chosen as the prototype of this group and was called FGB46. Therefore, we conclude that these mutants are affected in the *exb* locus, a finding that further supports the need for the TonB pathway for MccH47 uptake.

***atp* mutants.** Mutants from the third group were characterized by their high level of resistance to MccH47 and by their poor growth on rich and minimal glucose media. P1 transducing phage was grown on one of these mutants, and the lysate was used to infect BZB1011, selecting clones on LB plates supplemented with kanamycin. All of the resulting transductants were MccH47^r, thus confirming that the Tn*5* insertion, now called Tn*5 64*, was responsible for the MccH47^r phenotype. One of these transductants was named FGB64 and was used in further studies.

To identify the locus affected in FGB64 by the Tn*5 64* insertion, this mutation was cloned in vivo using the chimerical phage Mu*d*II4042, as described in Materials and Methods. Transductants of strain MC4100 Mu*cts*Ap^r should carry recombinant plasmids containing Mu*d*II4042 DNA, part or all of the Tn*5* sequence, and presumably, chromosomal DNA adjacent to the transposon in FGB64. Plasmids from three transductant clones were extracted and physically analyzed with *Hin*dIII (data not shown). Their restriction pattern indicated that a *Hin*dIII fragment of ca. 1,350 bp would contain one of the ends of the Tn*5* insertion. This fragment was cloned into the *Hin*dIII site of the pUC13 vector, and the recombinant plasmid obtained was used to sequence the ends of the insert. On one side, 170 nucleotides were read before reaching the Tn*5* end. This sequence was compared with those in databases using the program FASTA (25) and was found to correspond to the *atp* operon. The Tn*5* insertion was located after nucleotide 1038 of the reported *atp* sequences (41), interrupting the sixth codon of the *atpB* gene, which is the first gene of the *atp* operon coding for a polypeptide of the ATP synthase complex.

Mutants affected in the *atp* locus are impaired for oxidative phosphorylation since they encode abnormal or no ATP synthase. They can grow in most media, albeit poorly, thanks to substrate-level phosphorylation. It is known that deficient *atp* mutants are unable to grow in minimal media supplemented with a nonfermentable carbon source, as is the case with succinate (5). FGB64 and 11 other MccH47^r mutants from the third group were seeded on M63 succinate plates and did not grow, exhibiting an Atp$^-$ phenotype. The plasmid pBJC917, carrying the *atp* operon, was introduced by transformation into these 12 mutants. The strains constructed were MccH47^s and Atp$^+$, i.e., the *atp* operon complemented for both phenotypes. Thus, the *atp* locus appeared to be involved in conferring MccH47 sensitivity. Other *atp* strains previously described, such as CM1470 *recA* (40), which bears a deletion in the *atp* operon, were assayed with MccH47 and were found to be resistant.

The MccH47 structural gene *mchB* encodes a peptide precursor that possesses antibiotic activity of the same specificity as that of mature microcin. Cells transformed with the recombinant plasmid pMVD10 had been found to be nonviable (31). This plasmid carries an *mchB-phoA* gene fusion whose product retains antibiotic activity exerted as a toxic effect on the plasmid-bearing cells. pMVD10 was now used to transform four MccH47^r Atp$^-$ mutants from the third group, including FGB64, as well as BZB1011 (wild type), FGB11 (*tonB*), and FGB46 (*exb*), selecting the transformant clones on LB supplemented with chloramphenicol. Abundant colonies appeared in the experiments with mutant Atp$^-$ strains, while no clones grew in the remaining plates. These results showed that the *atp* mutants analyzed are resistant to the toxic intracellular effect exerted by the MccH47 precursor, besides being resistant to extracellular microcin. On the contrary, *tonB* and *exb* mutants only resisted MccH47 coming from outside, which is the expected phenotype for uptake mutants. Therefore, the presence of ATP synthase would be necessary for MccH47 action, although this complex does not appear to be involved in microcin uptake. The present results lead us to consider ATP synthase as the possible target of MccH47.

***cir*, *fiu*, and *fepA* mutants.** MccH47^r mutants so far isolated were not affected in any outer membrane determinant, a fact that could indicate that this antibiotic does not need to interact with a surface receptor as a first step in its uptake. However, a triple mutant receptor strain, H1876 (*cir fiu fepA*), was found to be completely resistant to MccH47. These genes code for iron-regulated outer membrane proteins which interact with the TonB pathway: FepA is the ferrienterochelin receptor, while Cir and Fiu are both receptors for the ferric complexes of the enterochelin-related compounds dihydroxybenzoylserine and dihydroxybenzoate; FepA also participates in dihydroxybenzoylserine recognition (15). In our case, mutants affected in one or two of these loci remained sensitive to MccH47, although a double-mutant *fiu cir* strain, H1728, displayed a slightly decreased sensitivity. These results suggest that MccH47 indeed interacts with outer membrane receptors but that any of the three catechol receptors, FepA, Fiu, or Cir, would be able to accomplish this function. This could explain our failure in the isolation of MccH47 receptor mutants.

Since production of FepA, Fiu, and Cir is induced under limited-iron conditions (10), the sensitivity of strain BZB1011 to MccH47 was assayed with and without the addition of the iron chelator EDDHA. The critical dilution method was employed as described in Materials and Methods. A fourfold increase in the sensitivity of BZB1011 was detected under low-iron conditions compared to results of the assay in the absence of EDDHA. This result is in accordance with the

finding that the iron-regulated catechol receptors are involved in uptake of MccH47.

The presented analyses on the MccH47 mode of action indicate that this peptide antibiotic probably starts its action by binding to the outer membrane receptor proteins FepA, Fiu, or Cir and then crosses the outer membrane through the TonB pathway. In addition, ATP synthase was found to be required for MccH47 action. *atp* mutants resisted both mature MccH47 proceeding from the external milieu and the intracellular toxic activity of the MccH47 precursor. This phenotype characterizes target mutants, pointing to the possibility that ATP synthase could be the subcellular structure where MccH47 exerts its final antibiotic effect. Previous results on MccH47 immunity, a function clearly assigned to an integral membrane peptide, are in accordance with a membrane localization of the MccH47 target (30).

Several antifungal natural products that inhibit the eukaryotic mitochondrial F_oF_1-ATP synthase have been described, including the well-known polyketide oligomycin (26, 32). However, there are no precedents of antibiotics targeted on the prokaryotic ATP synthase complex.

The results presented here suggest that ATP synthase could be the target of microcin H47. If this were the case, it would be the first description of an antibacterial effect exerted through interaction with this subcellular complex. More studies will be needed to test this hypothesis.

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REFERENCES

1. **Ahmer, B. M. M., M. G. Thomas, R. A. Larsen, and K. Postle.** 1995. Characterization of the *exbBD* operon of *Escherichia coli* and the role of ExbB and ExbD in TonB function and stability. J. Bacteriol. **177:**4742–4747.
2. **Azpiroz, M. F., E. Rodríguez, and M. Laviña.** 2001. The structure, function, and origin of the microcin H47 ATP-binding cassette exporter indicate its relatedness to that of colicin V. Antimicrob. Agents Chemother. **45:**969–972.
3. **Baquero, F., and F. Moreno.** 1984. The microcins. FEMS Microbiol. Lett. **23:**117–124.
4. **Berg, D. E.** 1977. Insertion and excision of a transposable kanamycin resistance determinant Tn*5*, p. 205–212. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Boogerd, F. C., L. Boe, O. Michelsen, and P. R. Jensen.** 1998. *atp* mutants of *Escherichia coli* fail to grow on succinate due to a transport deficiency. J. Bacteriol. **180:**5855–5859.
6. **Braun, M., H. Killmann, and V. Braun.** 1999. The β-barrel domain of FhuAΔ5–160 is sufficient for TonB-dependent FhuA activities of *Escherichia coli*. Mol. Microbiol. **33:**1037–1049.
7. **Braun, V., S. Gaisser, C. Herrmann, K. Kampfenkel, H. Killmann, and I. Traub.** 1996. Energy-coupled transport across the outer membrane of *Escherichia coli*: ExbB binds ExbD and TonB in vitro, and leucine 132 in the periplasmic region and aspartate 25 in the transmembrane region are important for ExbD activity. J. Bacteriol. **178:**2836–2845.
8. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K12: cross-resistance among colicins of group B. J. Bacteriol. **123:**96–101.
9. **de Lorenzo, V., and A. P. Pugsley.** 1985. Microcin E492, a low-molecular-weight peptide antibiotic which causes depolarization of the *Escherichia coli* cytoplasmic membrane. Antimicrob. Agents Chemother. **27:**666–669.
10. **Earhart, C. F.** 1996. Uptake and metabolism of iron and molybdenum, p. 1075–1090. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*, cellular and molecular biology. ASM Press, Washington, D.C.
11. **Fischer, E., K. Günter, and V. Braun.** 1989. Involvement of ExbB and TonB in transport across the outer membrane of *Escherichia coli*: phenotypic complementation of *exb* mutants by overexpressed *tonB* and physical stabilization of TonB by Exb. J. Bacteriol. **171:**5127–5134.
12. **Gaggero, C., F. Moreno, and M. Laviña.** 1993. Genetic analysis of microcin H47 antibiotic system. J. Bacteriol. **175:**5420–5427.
13. **García-Bustos, J. F., N. Pezzi, and E. Méndez.** 1985. Structure and mode of action of microcin 7, an antibacterial peptide produced by *Escherichia coli*. Antimicrob. Agents Chemother. **27:**791–797.
14. **Groisman, E. A., B. A. Castilho, and M. J. Casadaban.** 1984. *In vivo* DNA cloning and adjacent gene fusing with a mini-Mu-*lac* bacteriophage containing a plasmid replicon. Proc. Natl. Acad. Sci. USA **81:**1480–1483.
15. **Hantke, K.** 1990. Dihydroxybenzolyserine—a siderophore for *E. coli*. FEMS Microbiol. Lett. **67:**5–8.
16. **Hantke, K., and V. Braun.** 1975. Membrane receptor-dependent iron transport in *Escherichia coli*. FEBS Lett. **49:**301–305.
17. **Jack, R. W., and G. Jung.** 2000. Lantibiotics and microcins: polypeptides with unusual chemical diversity. Curr. Opin. Chem. Biol. **4:**310–317.
18. **Killmann, H., and V. Braun.** 1994. Energy-dependent receptor activities of *Escherichia coli* K-12: mutated TonB proteins alter FhuA receptor activities to phages T5, T1, phi 80 and to colicin M. FEMS Microbiol. Lett. **119:**71–76.
19. **Kolter, R., and F. Moreno.** 1992. Genetics of ribosomally synthesized peptide antibiotics. Annu. Rev. Microbiol. **46:**141–164.
20. **Laviña, M., A. P. Pugsley, and F. Moreno.** 1986. Identification, mapping, cloning and characterization of a gene (*sbmA*) required for microcin B17 action on *Escherichia coli* K12. J. Gen. Microbiol. **132:**1685–1693.
21. **Laviña, M., C. Gaggero, and F. Moreno.** 1990. Microcin H47, a chromosome-encoded microcin antibiotic of *Escherichia coli*. J. Bacteriol. **172:**6585–6588.
22. **Leach, D., and N. Symonds.** 1979. The isolation and characterization of a plaque-forming derivative of bacteriophage Mu carrying a fragment of Tn*3* conferring ampicillin resistance. Mol. Gen. Genet. **172:**179–184.
23. **Mayr-Harting, A., A. J. Hedges, and R. C. W. Berkeley.** 1972. Methods for studying bacteriocins. Methods Microbiol. **7A:**315–422.
24. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
26. **Penefsky, H. S.** 1985. Mechanism of inhibition of mitochondrial adenosine triphosphatase by dicyclohexylcarbodiimide and oligomycin: relationship to ATP synthesis. Proc. Natl. Acad. Sci. USA **82:**1589–1593.
27. **Postle, K.** 1990. TonB and the Gram-negative dilemma. Mol. Microbiol. **4:**2019–2025.
28. **Pugsley, A. P.** 1985. *Escherichia coli* K12 strains for use in the identification and characterization of colicins. J. Gen. Microbiol. **131:**369–376.
29. **Pugsley, A. P., and B. Oudega.** 1987. Methods for studying colicins and their plasmids, p. 105–161. *In* K. G. Hardy (ed.), Plasmids, a practical approach. IRL Press, Oxford, United Kingdom.
30. **Rodríguez, E., and M. Laviña.** 1998. Genetic analysis of microcin H47 immunity. Can. J. Microbiol. **44:**692–697.
31. **Rodríguez, E., C. Gaggero, and M. Laviña.** 1999. The structural gene for microcin H47 encodes a peptide precursor with antibiotic activity. Antimicrob. Agents Chemother. **43:**2176–2182.
32. **Salomon, A. R., D. W. Voehringer, L. A. Herzenberg, and C. Khosla.** 2001. Apoptolidin, a selective cytotoxic agent, is an inhibitor of FoF1-ATPase. Chem. Biol. **8:**71–80.
33. **Salomón, R. A., and R. N. Farías.** 1992. Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. J. Bacteriol. **174:**7428–7435.
34. **Salomón, R. A., and R. N. Farías.** 1993. The FhuA protein is involved in microcin 25 uptake. J. Bacteriol. **175:**7741–7742.
35. **Salomón, R. A., and R. N. Farías.** 1995. The peptide antibiotic microcin 25 is imported through the Ton B pathway and the SbmA protein. J. Bacteriol. **177:**3323–3325.
36. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
37. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
38. **Vizán, J. L., C. Hernández-Chico, I. del Castillo, and F. Moreno.** 1991. The peptide antibiotic microcin B17 induces double-strand cleavage of DNA mediated by *E. coli* DNA gyrase. EMBO J. **10:**467–476.
39. **von Meyenburg, K., B. B. Jorgensen, and B. van Deurs.** 1984. Physiological and morphological effects of overproduction of membrane-bound ATP synthase of *Escherichia coli* K-12. EMBO J. **3:**1791–1797.
40. **von Meyenburg, K., B. B. Jorgensen, J. Nielsen, and F. G. Hansen.** 1982. Promoters of the *atp* operon coding for the membrane-bound ATP synthase of *Escherichia coli* mapped by Tn*10* insertion mutations. Mol. Gen. Genet. **188:**240–248.
41. **Walker, J. E., M. Saraste, and N. J. Gay.** 1984. The *unc* operon, nucleotide sequence, regulation, and structure of ATP-synthase. Biochim. Biophys. Acta **768:**164–200.
42. **Yang, C. C., and J. Konisky.** 1984. Colicin V-treated *Escherichia coli* does not generate membrane potential. J. Bacteriol. **158:**757–759.