Purification and Characterization of Staphylococcin BacR1, a Broad-Spectrum Bacteriocin

SCOTT S. CRUPPER, ALLEN J. GIES, AND JOHN J. IANDOLO*

*Department of Microbiology and Immunology, University of Oklahoma
Health Sciences Center, Oklahoma City, Oklahoma 73190*

Received 29 January 1997/Accepted 19 August 1997

The bacteriocin BacR1 was purified from culture supernatant of *Staphylococcus aureus* UT0007 by sequential ammonium sulfate precipitation, cation-exchange chromatography, and C₄ reverse-phase chromatography steps. Mass spectrographic analysis indicated that the purified peptide has a molecular mass of 3,338 Da. It is resistant to environmental conditions, retaining full biological activity after exposure to pH extremes (pHs 3 to 11), heating at 95°C for 15 min, and exposure to strong chaotropic agents. BacR1 was destroyed with a complete loss of biological activity after digestion with trypsin and proteinase K. Amino acid sequence analysis revealed a high concentration of Asx, Gly, and Pro residues and a high proportion of hydrophobic amino acids. The peptide is bactericidal and kills in a dose-dependent manner, but it does not lyse log-phase cells of *Corynebacterium renale*, the routine indicator organism for bacteriocin assay. A specific receptor for binding was detected on sensitive cells but not on insensitive cells. Competition assays showed that UV-inactivated cells could protect susceptible cells from antibacterial action. A partial inhibitory spectrum revealed that organisms from the following genera are susceptible: *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Haemophilus*, *Bordetella*, *Moraxella*, *Pasteurella*, *Neisseria*, and *Bacillus*.

The colicins produced by the gram-negative *Enterobacteriaceae* (11) generally exhibit (i) a narrow range of activity against closely related species, (ii) a biologically active protein component, (iii) bactericidal activity, (iv) attachment to specific cell surface receptors, (v) plasmid-borne genetic determinants, and (vi) specific immunity mechanisms to avoid cell suicide (25).

Bacteriocins are colicin-like compounds produced by gram-positive bacteria (8, 15). They generally exhibit a broad range of activity against gram-positive bacteria, and in some cases gram-negative species are also inhibited. Host cell immunity, although present, is less well defined (i.e., in many cases immunity is documented but not investigated) (15). The genes for bacteriocin production are usually arranged in an operon-like structure and are carried on plasmids. In addition, the operons containing the structural genes of the bacteriocins nisin (6) and epidermin (1) also encode gene products responsible for post-translational modifications, processing, cellular export, and host cell immunity.

Chemically, bacteriocins usually consist of a protein moiety that is responsible for their bactericidal mechanism of action. Most are small cationic peptides that are relatively heat stable, sensitive to proteolytic enzymes, nonantigenic, and hydrophobic (15, 25). High-molecular-weight bacteriocins that are more complex and consist of proteins complexed with lipids and/or carbohydrates have also been identified (15).

Although the majority of research has focused on bacteriocins produced by the lactic-acid bacteria, due to their potential for food preservation (14), there are several reports of the existence of staphylococcal bacteriocins (4, 5, 9, 12, 16, 21, 23). Most of the characterized antimicrobial compounds from *Staphylococcus aureus* are associated with bacteriophage lytic group II, phage type 71 strains, and they comprise a group of molecules with very diverse chemical compositions and modes

of action. Examples include the staphylococcins 412 (9), 462 (12), C55 (5), and BacR1 (23).

The genetic determinants for staphylococcin BacR1 production and immunity are carried on large plasmids generally associated with the production of exfoliative toxin B (19, 28). BacR1 has only been partially purified by ammonium sulfate precipitation and Sephadex G-50 gel filtration chromatography (23). Although its molecular weight has not been determined, some of the general properties of BacR1 have been described (23). The activity in crude preparations is heat stable and bactericidal, and a partial inhibitory spectrum has revealed that organisms from the genera *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Bacillus* are sensitive. In this report, we describe the purification and characterization of BacR1. We have determined its molecular weight, amino acid composition, and mode of action. Purified BacR1 has a broad spectrum of activity against both gram-positive and gram-negative organisms, making it a possible therapeutic agent against pathogenic organisms.

MATERIALS AND METHODS

Bacterial strains. *S. aureus* UT0007 (23) contains the plasmid pRW001, which carries the genetic determinants for BacR1. It was maintained on Trypticase soy agar but was propagated in 2× YT liquid medium (5 g of NaCl per liter, 16 g of Bacto-Tryptone per liter, 10 g of Bacto Yeast extract per liter) at 37°C with shaking at 220 rpm for the production of BacR1. *Corynebacterium renale* ATCC 19412, the routine indicator strain for monitoring bacteriocin production, was maintained in brain heart infusion broth containing 0.3% Tween 80 (BHT-80).

Bacteriocin assay. Bacteriocin production was determined as described previously by either dilution analysis with a modified agar well diffusion assay or a tube dilution assay (18, 24).

Dilution analysis on agar plates was carried out by seeding 20 ml of BHT-80 agar with 100 µl of an overnight culture of *C. renale* (ca. 10⁸ bacteria/ml). Holes (3 mm in diameter) were aseptically bored into the agar with a gel punch, and 25 µl of a serially diluted bacteriocin preparation was pipetted into the wells. The plates were examined for zones of inhibition after overnight incubation at 37°C. Biological activity units (AU) were recorded as the reciprocal of the highest dilution giving a zone of inhibition, and the data are expressed as AU per milliliter. For the tube dilution assay, an overnight culture of *C. renale* was used to inoculate fresh BHT-80 to an initial *A*₅₅₀ of 0.05 and incubated at 37°C with shaking at 220 rpm for 3 h to reach early log phase. This culture was diluted 1:100 into 0.4 ml of BHT-80 which contained 10 µl of serially diluted BacR1, and the

* Corresponding author. Phone: (405) 271-2133. Fax: (405) 271-3117.

FIG. 1. (A) Growth of *S. aureus* UT0007 and BacR1 production. An overnight culture of UT0007 was used to inoculate 2× YT media to an initial A_{550} of 0.05, and the culture was incubated at 37°C with shaking at 220 rpm. At intervals, the log number of cells (●) was determined by plate counts, and BacR1 activity (bars) was measured by dilution analysis on *C. renale* plates. (B) Assay of serially diluted culture-free supernatants collected between 8 and 24 h and incubated at 37°C overnight.

tubes were incubated at 37°C with shaking at 220 rpm for 5 h. Growth of the culture was determined by measuring the difference in A_{550} between the BacR1 culture and control cultures which did not receive BacR1. The MIC₅₀ was calculated as the concentration of bacteriocin that caused a 50% inhibition of growth.

BacR1 purification. An overnight culture of *S. aureus* UT0007 was used to inoculate 1 liter of 2× YT media to an initial A_{550} of 0.05. The culture was incubated at 37°C with shaking at 220 rpm for 18 h, and the cells were then removed by centrifugation at $5,000 \times g$ for 10 min. The culture supernatant was filter sterilized by passage through a 0.45- μ m-pore-size membrane filter. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to 60% saturation at room temperature, and the solution was stirred for approximately 20 min until all of the ammonium sulfate had dissolved. A floating pellicle composed of protein and lipid was removed, and the suspension was centrifuged at $9,000 \times g$ for 20 min at 4°C. The supernatant was discarded and the precipitate was dissolved in a minimal volume of 25% acetonitrile–10 mM sodium phosphate, (pH of mixture, 3.0). After centrifugation at $9,000 \times g$ for 10 min at 4°C to remove insoluble protein, the sample was bound to a CM 300 cation-exchange column (250 by 10 mm) (SynChrom, Inc., Lafayette, Ind.) and eluted with an increasing gradient of NaCl in acetonitrile at a flow rate of 1 ml/min. Buffer A consisted of 25% acetonitrile–10 mM sodium phosphate (pH of mixture, 3.0), and buffer B was composed of buffer A and 1 M NaCl. The gradient consisted of 0 M NaCl over the first 20 min, and the concentration was increased to 1 M over the next 35 min (0 to 20 min, 0% buffer B; 20 to 55 min, 100% buffer B). Fractions were collected, dried by evaporation, resuspended in 0.5 ml of distilled water, and analyzed for activity. Fractions containing peak bacteriocin activity were pooled, concentrated by evaporation, and then passed over a C₄ reverse-phase chromatography column (250 by 4.6 mm) (Vydac, Hesperia, Calif.) at flow rates of 1 ml/min. Buffer A1 consisted of 25% acetonitrile–0.1% trifluoroacetic acid, and buffer B1 was 85% acetonitrile–0.1% trifluoroacetic acid. The gradient consisted of 25% acetonitrile for the first 10 min, 55% acetonitrile over the next 30 min, and finally 85% acetonitrile over the final 5 min (0 to 10 min, 0% buffer B; 10 to 40 min, 50% buffer B; 40 to 45 min, 100% buffer B). Fractions with bacteriocin activity were concentrated by evaporation, and final purification was achieved by a second cycle of C₄ reverse-phase chromatography with a shallower acetonitrile gradient. The running conditions consisted of 25% acetonitrile for the first 5 min, 37% acetonitrile over the next 5 min, 43% acetonitrile over the next 14 min, 55% acetonitrile over the next 6 min, and finally 85% acetonitrile over the final 10 min (0 to 5 min, 0% buffer B1; 5 to 10 min, 20% buffer B1; 10 to 24 min, 30% buffer B1; 24 to 30 min, 50% buffer B1; and 30 to 40 min, 100% buffer B1). The peak fraction of BacR1 activity was dried by evaporation and resuspended in distilled water, and the total activity and protein content were determined.

SDS-PAGE and bioassay. Analysis of purified BacR1 preparations was carried out by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (17) on 10 to 20% monomer gradient gels. After electrophoresis, gels were either silver stained (3) or bioassayed by a modification of the method of Bhunia et al. (2). The gels were soaked in a solution composed of 20% isopropanol and 10% acetic acid for 2 h to remove the SDS, followed by rinsing in distilled water for 4 h. The washed gels were placed on a BHT-80 agar plate and overlaid with BHT-80 agar (0.7%) containing *C. renale* at a concentration of 10^8 organisms/ml. The plate-gel combination was incubated at 37°C overnight prior to examination for zones of clearing over the bands of BacR1.

Analytical methods. To precisely determine the size of purified BacR1, the samples were analyzed on a Lasermat matrix-assisted laser desorption mass spectrometer at the Kansas State University Biotechnology facility. Approxi-

mately 1 pmol of BacR1 was mixed with 1 μ l of substance P (10 mg/ml) and 1 μ l of alpha-cyano-4-hydroxycinnamic acid (10 mg/ml). Analysis of the sample was carried out with the Lasermat settings as follows: laser power, 35; accelerating voltage, 20,020 V; and gain, 31 mV. Amino acid analysis was performed with an Applied Biosystems model 420A amino acid analysis system at the Kansas State University Biotechnology Facility. Protein determinations were accomplished either by measuring the A_{280} of the solution or with a DC protein assay kit (Bio-Rad), with bovine serum albumin as a standard for both assays.

RESULTS AND DISCUSSION

In this communication we describe the biological properties of the purified staphylococcal BacR1. We have purified BacR1 to homogeneity and have confirmed that it possesses the necessary biological and physical properties to be classified as a bacteriocin.

The propagation of strain UT0007 in 2× YT media resulted in maximal levels of BacR1 being detected after 7 h of growth (Fig. 1A), but the level remained stable thereafter. To assess if this was due to protein degradation, filter-sterilized culture supernatants of UT0007 taken between 8 and 24 h of incubation were incubated at 37°C and assayed periodically (Fig. 1B). The titers of BacR1 remained constant throughout, suggesting that turnover was not responsible for the lack of further accumulation. Although we have not directly examined de novo synthesis, it appears that a further conclusion to be drawn from these data is that production of BacR1 may be tightly regulated, because accumulation ceases after approximately 7 h. The abrupt shutdown of synthesis might indicate that environmental factors operate via a signal transduction mechanism similar to that recently reported for both nisin (7) and epider-

TABLE 1. Purification of BacR1

Purification step	Total protein (mg)	Total activity (AU) ^a	Sp act (AU/mg) ^b	Yield (%)	Purification (fold)
Supernatant (1 liter)	2,100	320,000	152	100	1
Ammonium sulfate precipitate	211.4	266,240	1,259	83.2	8.3
Cation exchange	4.11	28,620	6,963	8.9	45.8
C ₄ reverse phase	0.24	3,994	16,642	1.25	109.5
C ₄ reverse phase	0.04	1,498	37,450	0.47	246.4

^a Determined by plate dilution on a *C. renale* plate.

^b Specific activity is total activity divided by the total protein.

FIG. 2. Elution profile of BacR1 activity after C_4 reverse-phase chromatography with a 1%/min acetonitrile gradient. Pooled samples from the CM 300 cation-exchange column were fractionated by an increasing gradient of acetonitrile as described in Materials and Methods. The absorbance at 220 nm of each 1-ml fraction was measured, and then the fraction was assayed for BacR1 activity by dilution analysis on *C. renale* plates. Fractions 25 and 26, which contained BacR1 activity, were pooled.

min (22). However, these conclusions remain tentative until the organization of the operon is known and expression can be examined by gene transcription analysis and radioisotopic pulse-chase experiments. At this time, we do not have gene probes to carry out Northern analysis of transcription. Moreover, pulse-chase experiments are hampered because BacR1 is not produced in minimal medium and in rich medium (i.e., $2\times$ YT) sufficient label incorporation for this analysis cannot be attained.

The prolonged stability of BacR1 in culture is similar to that of other bacteriocins (4, 10, 21, 26), and it allowed the culture to be harvested at convenience without loss of activity. BacR1 activity was precipitated from culture supernatants by adding solid ammonium sulfate to reach 60% saturation. No measurable increase in activity was observed at higher salt concentrations, and this step provided a convenient way to concentrate large sample volumes.

The ammonium sulfate precipitates were applied to ion-exchange columns. Chromatography was carried out in the presence of 25% acetonitrile to minimize hydrophobic interactions with the column matrix and other proteins. BacR1 did not bind to either an anion- or a cation-exchange column and eluted from both over several fractions. However, cation-exchange chromatography at pH 3 removed a significant amount of contaminating protein and resulted in the best overall purification (Table 1).

Final purification of concentrated, pooled samples from the cation-exchange step was achieved by high-performance liquid chromatography with a C_4 reverse-phase column (Fig. 2) and resulted in a significant increase in specific activity (Table 1). When the samples were reapplied to the C_4 column under the same running conditions, a single homogeneous peak was detected (data not shown). The final purification resulted in a

specific activity of 37,450 AU/mg, with a total yield of 0.47% (Table 1). SDS-PAGE gels containing this fraction were not stainable with either Coomassie blue or silver, but BacR1 could be localized by biological activity when overlaid with *C. renale* in soft agar (Fig. 3). The molecular mass of BacR1 was estimated from this gel to be about 3,500 Da. More precise measurement by mass spectrographic analysis of the same

FIG. 3. Overlay of purified BacR1 on a 10 to 20% monomer gradient gel. Results of SDS-PAGE of BacR1 overlaid with BHT-80 top agar seeded with *C. renale* are shown. The zone of clearing represents the antimicrobial activity of BacR1.

TABLE 2. Amino acid composition of BacR1

Amino acid	No. of residues
Asx.....	3
Glx.....	2
Ser.....	2
Gly.....	12
His.....	1
Arg.....	0
Thr.....	0
Ala.....	4
Pro.....	4
Tyr.....	1
Val.....	1
Met.....	1
Ile.....	1
Leu.....	2
Phe.....	1
Lys.....	2
Cys.....	0
Trp.....	ND ^a

^a ND, not determined.

preparation showed a homogenous single peak with a molecular mass of 3,338 Da. We have used automated Edman degradation to partially sequence BacR1 and have determined that the peptide has the N-terminal sequence L-G-G-A-A-I. Although it is theoretically feasible to sequence an entire peptide of this molecular weight, we have been unable (in two separate attempts) to extend the sequence beyond the hexamer reported. However, we have been able to use this information to generate DNA probes to identify a fragment of DNA containing the genes for BacR1, and we are pursuing the sequence at the DNA level.

Purified BacR1 is resistant to a wide range of environmental conditions, including extremes of temperature, and maintained full activity even after exposure for 15 min to temperatures from -20 to 95°C and pHs from 3 to 11. Strikingly, even

TABLE 3. Inhibitory spectrum of BacR1

Test organism	Susceptibility ^a
<i>Escherichia coli</i>	-
<i>Pseudomonas aeruginosa</i>	-
<i>Klebsiella pneumoniae</i>	-
<i>Salmonella typhimurium</i>	-
<i>Actinobacillus pleuropneumoniae</i>	-
<i>Enterococcus faecalis</i> JH2-2.....	-
<i>Staphylococcus aureus</i> RN4220.....	+
<i>Staphylococcus aureus</i> 8325.....	+
<i>Staphylococcus aureus</i> 502A.....	+
<i>Staphylococcus intermedius</i>	+
<i>Streptococcus suis</i>	+
<i>Streptococcus porcinus</i>	+
<i>Streptococcus equi</i>	-
<i>Streptococcus canis</i>	-
<i>Corynebacterium renale</i>	+
<i>Corynebacterium diphtheriae</i>	+
<i>Corynebacterium pseudotuberculosis</i> Whetten 1.....	+
<i>Haemophilus somnus</i>	-
<i>Haemophilus parasuis</i>	+
<i>Bordetella pertussis</i>	+
<i>Bordetella bronchiseptica</i>	+
<i>Moraxella bovis</i>	+
<i>Pasteurella multocida</i>	+
<i>Neisseria meningitidis</i> ^b	+
<i>Neisseria gonorrhoeae</i> ^b	+
<i>Bacillus subtilis</i> Marburg ^c	+
<i>Bacillus licheniformis</i> FD01 ^c	+
<i>Bacillus megaterium</i> ATCC 19213 ^c	+

^a Susceptibility to BacR1 was determined by bactericidal assays described in Materials and Methods.

^b As noted in reference 18.

^c As noted in reference 20.

FIG. 4. Dose-dependent killing of *C. renale* by BacR1. Cultures of *C. renale* (10^8 cells) were incubated with purified BacR1 at various concentrations. After incubation at 37°C with shaking for 5 h, the A_{550} was measured. Percent survival of *C. renale* (●) was determined by dividing the A_{550} of the *C. renale* cultures containing BacR1 by the A_{550} of the cultures without BacR1.

prolonged exposure to a temperature of 95°C had no effect on the biological activity of the molecule. Moreover, exposure for 1 h to 6 M urea, 1 M NaCl, or up to 1 mg each of DNase, RNase, and lysostaphin per ml had no effect on biological activity. However, exposure to 1 mg of trypsin or proteinase K per ml completely destroyed biological activity within 15 min. This sensitivity to proteolytic enzymes confirms the proteinaceous nature of the molecule, and amino acid analysis revealed a high molar concentration of hydrophobic amino acids, such as Gly, Ala, Pro, and Leu (Table 2). The hydrophobicity of BacR1 could account for its association with other cellular proteins throughout the various purification steps and may significantly affect yield.

BacR1 kills *C. renale* in a dose-dependent fashion, exhibiting 50% lethality at approximately 160.2 ng/ml for a population of 10^8 cells/ml (Fig. 4). Furthermore, the killing is rapid and occurs in the absence of cell lysis, as evidenced by the data in Fig. 5. When 10^8 cells/ml were exposed to 2,670 ng of BacR1 (100 AU), the absorbance of the suspension did not change, while the absorbance of an identical control culture increased (Fig. 5A). However, when antibacterial activity was assessed by viable-cell analysis (Fig. 5B), the BacR1-treated suspension showed a rapid decline in the number of viable cells. No viable cells were detected after 120 min of exposure to BacR1. These data show that BacR1 is bactericidal, but because the absorbance of the culture remains constant, it does not appear to lyse cells of *C. renale* or of other strains tested. Moreover, the cells do not recover when the bacteriocin is removed, as would be observed with a bacteriostatic agent.

Interaction of cells with BacR1 results in its being tightly bound by susceptible cells which quantitatively remove it from

FIG. 5. Effect of BacR1 on the viability of *C. renale*. Cultures of *C. renale* (10^8 cells) were incubated with 100 AU of BacR1 per ml, and samples were removed at the times indicated. Absorbance at 550 nm (A) and viable cell counts (B) were determined for control cells (●) and cells treated with BacR1 (■).

solution. Resistant cells do not adsorb BacR1 under the same conditions, and full potency of the solution is maintained. These data suggest that binding to susceptible cells may involve specific receptors. To investigate this possibility, we determined whether UV- and heat-killed *C. renale* would compete with live cells in the binding of bacteriocin. The data presented in Fig. 6 show that killing by 100 AU of BacR1 per ml was inhibited by the inclusion of excess UV-irradiated *C. renale* cells in the assays. At a concentration of 20 $\mu\text{g/ml}$, complete protection was observed. In contrast, UV-irradiated cells of *Escherichia coli* that are resistant to BacR1 do not afford protection to *C. renale*. Similar results were also obtained with heat-killed cells, but at 100-fold-higher levels of *C. renale*, suggesting that the putative receptor might be heat denaturable. In additional experiments (data not shown), we have also determined that the binding of bacteriocin is not inhibited by NaCl concentrations as high as 600 mM, whereas this concentration of salt inhibits bacteriocins that do not rely on specific receptors (15). Lastly, binding is pH dependent, with the maximum level occurring at pH 8.

Both gram-positive and gram-negative organisms are sensitive to the lethal effects of BacR1 (Table 3). These data show

that pathogens such as *S. aureus*, *Streptococcus suis*, *Bordetella bronchiseptica*, and *Pasteurella multocida* are all killed. However, an unusual species specificity was noticed, because not all strains tested from the genera *Corynebacterium*, *Bordetella*, *Staphylococcus*, *Neisseria*, and *Bacillus* were killed. This result is not understood at present. One of the organisms that are more sensitive to BacR1 is *Moraxella bovis*. When tested, strains of this organism showed zones of inhibition that were similar in size to those of *C. renale* and two- to fourfold larger than those of other susceptible organisms. As an example of the potential use of BacR1 as a therapeutic agent, the treatment of infectious bovine keratoconjunctivitis caused by *M. bovis* may be a reasonable use for this antimicrobial. This serious disease of the eye in cattle (13) causes economic losses which approach \$150 million annually (27). The broad-spectrum bacteriocin BacR1 may therefore prove beneficial as a possible alternative therapeutic agent for treatment of various bacterial infections.

ACKNOWLEDGMENT

This work was supported by grant AI-17474 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. Augustin, J., R. Rosenstein, B. Wieland, U. Schneider, N. Schnell, G. Engelke, K.-D. Entian, and F. Götz. 1992. Genetic analysis of epidermin biosynthetic genes and epidermin-negative mutants of *Staphylococcus epidermidis*. *Eur. J. Biochem.* **204**:1149–1154.
2. Bhunia, A. K., M. C. Johnson, and B. Ray. 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Ind. Microbiol.* **2**:319–322.
3. Blum, H., H. Berei, and H. J. Gross. 1987. Improved silver staining of plant proteins, RNA, and DNA in polyacrylamide gels. *Electrophoresis* **8**:93–99.
4. Crupper, S. S., and J. J. Iandolo. 1996. Purification and partial characterization of a novel antibacterial agent (Bac1829) produced by *Staphylococcus aureus* KSI1829. *Appl. Environ. Microbiol.* **62**:3171–3175.
5. Dajani, A. S., E. D. Gray, and L. W. Wannamaker. 1970. Effect of bactericidal substance from *Staphylococcus aureus* on group A streptococci. I. Biochemical alterations. *Infect. Immun.* **1**:485–490.
6. Engelke, G., Z. Gutowski-Eckel, M. Hammelmann, and K.-D. Entian. 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the NisB protein. *Appl. Environ. Microbiol.* **58**:3730–3743.
7. Engelke, G., Z. Gutowski-Eckel, P. Kiesau, K. Siegers, M. Hammelmann, and K.-D. Entian. 1994. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. *Appl. Environ. Microbiol.* **60**:814–825.
8. Frédericq, P. 1948. Actions antibiotiques réciproques chez les *Enterobacteriaceae*. *Rev. Belge. Pathol. Med. Exp.* **19**(Suppl. 4):1–107.
9. Gagliano, V. J., and R. D. Hinsdill. 1970. Characterization of a *Staphylococ-*

FIG. 6. Competition for BacR1 binding by UV-irradiated cells of *C. renale* and *E. coli*. Increasing amounts of UV-irradiated cells were mixed with viable *C. renale* cells, and survival was measured. ■, untreated *C. renale*; ●, UV-irradiated *C. renale*; ▲, UV-irradiated *E. coli*. There are approximately 10^8 CFU/ μg of cells used in the protection assay, and BacR1 was present at 100 AU/ml.

- cus aureus* bacteriocin. J. Bacteriol. **104**:117–125.
10. González, B., P. Arca, B. Mayo, and J. E. Suárez. 1994. Detection, purification, and partial characterization of plantaricin C, a bacteriocin produced by a *Lactobacillus plantarum* strain of dairy origin. Appl. Environ. Microbiol. **60**:2158–2163.
 11. Gratia, A. 1925. Sur un remarquable exemple d'antagonisme entre souches de colibacille. C. R. Soc. Biol. **93**:1040–1041.
 12. Hale, E. M., and R. D. Hinsdill. 1973. Characterization of a bacteriocin from *Staphylococcus aureus* strain 462. Antimicrob. Agents Chemother. **4**:634–640.
 13. Hughes, D. E., and G. W. Pugh, Jr. 1970. A five-year study of bovine infectious keratoconjunctivitis in a beef herd. J. Am. Vet. Med. Assoc. **157**:443–451.
 14. Hurst, A. 1981. Nisin. Adv. Appl. Microbiol. **27**:85–123.
 15. Jack, R. W., J. R. Tagg, and B. Ray. 1995. Bacteriocins of gram-positive bacteria. Microbiol. Rev. **59**:171–200.
 16. Kader, O. A., H.-G. Sahl, and H. Brandis. 1984. Isolation and mode of action of staphylococcin-like substance against gram-positive and gram-negative bacteria. J. Gen. Microbiol. **130**:2291–2300.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
 18. Mayr-Harting, A., A. H. Hedges, and R. C. W. Berkeley. 1972. Methods for studying bacteriocins. Methods Microbiol. **7A**:315–422.
 19. Melish, M. E., and L. A. Glasgow. 1971. Staphylococcal scalded skin syndrome: the expanded clinical syndrome. J. Pediatr. **78**:958–967.
 20. Morriss, D. M., J. W. Lawson, and M. Rogolsky. 1978. Effect of a staphylococin on *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **14**:218–223.
 21. Nakamura, T., N. Yamazaki, H. Taniguchi, and S. Fujimura. 1983. Production, purification, and properties of a bacteriocin from *Staphylococcus aureus* isolated from saliva. Infect. Immun. **39**:609–614.
 22. Peschel, A., J. Augustin, T. Kupke, S. Stefanovic, and F. Gotz. 1993. Regulation of epidermin biosynthetic genes by EpiQ. Mol. Microbiol. **9**:31–39.
 23. Rogolsky, M., and B. B. Wiley. 1977. Production and properties of a staphylococin genetically controlled by the staphylococcal plasmid for exfoliative toxin synthesis. Infect. Immun. **15**:726–732.
 24. Tagg, J. R., and A. R. McGiven. 1971. Assay system for bacteriocins. Appl. Microbiol. **21**:943.
 25. Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. **40**:722–756.
 26. Toba, T., S. K. Samant, E. Yoshioka, and T. Itoh. 1991. Reuterin 6, a new bacteriocin produced by *Lactobacillus reuteri* LA6. Lett. Appl. Microbiol. **13**:281–286.
 27. U.S. Department of Agriculture. 1976. Control of cattle diseases: infectious, non-infectious, and parasitic, p. 35–36. Agricultural Research Science National Research Program No. 20420. U.S. Department of Agriculture, Washington, D.C.
 28. Warren, R. L. 1980. Exfoliative toxin plasmids of bacteriophage group 2 *Staphylococcus aureus*: sequence homology. Infect. Immun. **30**:601–606.