JOURNAL OF BACTERIOLOGY, Oct. 1972, p. 235-242

Vol. 112, No. 1
Printed in U.S.A.

Production and Purification of a *Staphylococcus epidermidis* Bacteriocin

A. M. JETTEN, G. D. VOGELS, AND F. DE WINDT

Laboratory of Microbiology, Faculty of Science, University of Nijmegen, Nijmegen, The Netherlands

Received for publication 29 December 1971

Liquid cultures of *Staphylococcus epidermidis* 1580 contained rather small amounts of a bacteriocin, staphylococcin 1580, which was found both in the supernatant fluid and in the cell pellet. It could be extracted from the cells with 5% NaCl solution. The staphylococcin production could not be induced by ultraviolet irradiation or treatment with mitomycin C. Bacteria grown on semi-solid medium produced a much larger amount of the compound with a high specific activity. The staphylococcin was purified by ammonium sulfate precipitation, ultracentrifugation, and chromatography on Sephadex columns. The purified material was homogeneous on polyacrylamide gel electrophoresis. The molecular weight was between 150,000 and 400,000. The bacteriocin was composed of protein, carbohydrate, and lipid and consisted of subunits exhibiting a molecular weight of about 20,000.

Bacteriocins are high-molecular-weight bactericidal substances produced by various species of bacteria and are active against the same and related species. The specificity of their action and their nature distinguish them from most of the "classical" antibiotics. Many bacteriocins have been described and classified (23, 32, 35), but only a few of them have been studied in detail. The best studied of the bacteriocins are the colicins, bacteriocins produced by certain strains of *Enterobacteriaceae*.

Investigations of the chemical nature of bacteriocins have shown them to be a heterogeneous group of substances ranging from simple proteins (7, 13, 17, 20) and proteins complexed with carbohydrates and lipids (22, 24, 26) to particles resembling phages (18, 25, 30, 40).

The lethal action of a bacteriocin on sensitive bacterial cells seems to occur in two phases, namely, initial combination of the bacteriocin with specific receptor sites located on the cell surface followed by effect on an intracellular biochemical target via the mediation of a specific transmission system (19, 31).

The ability to synthesize bacteriocin depends upon the presence of a bacteriocinogenic factor. Certain bacteriocinogenic factors have been identified as extrachromosomal elements (plasmids) (3, 38).

The staphylococcins, bacteriocins produced by staphylococci, were first described by Frederique in 1946 (9). He distinguished several different staphylococcins on the basis of their inhibition spectrum. Probably because of the difficulty of producing (1, 2, 10) and isolating (21, 27, 34) many staphylococcins in large amounts, little is known about their nature, genetics, and mode of action (6, 28). Recent studies of Dajani et al. (4, 5) and Gagliano and Hinsdill (10) described the isolation and purification of two different staphylococcins. They have shown that, unlike many other bacteriocins, the production of staphylococcin seems not to be induced by ultraviolet irradiation or treatment with mitomycin C.

This study deals with the production and purification of a bacteriocin produced by *Staphylococcus epidermidis*. This bacteriocin is different from the two staphylococcins just mentioned.

MATERIALS AND METHODS

Microorganisms. The staphylococcin-producing strain *S. epidermidis* 1580 was obtained from T. Lachowicz of the Institute of Hygiene and Epidemiology, Krakow, Poland. The strain is coagulase negative, does not ferment mannitol, and is not hemolytic. *S. aureus* Oxford 209 P was used as indicator strain. Both strains were grown on Trypticase soy agar and were subcultured once a week.

Bacteriocin assay. Staphylococcin was determined by the method of Reeves (35) with slight modifications. One milliliter of serial dilutions of the bacteriocin was mixed in sterile tubes with 1 ml of Trypticase soy broth (TSB) and 10^7 bacteria from an exponential-phase culture of the indicator strain. Subsequently, the tubes were incubated at 37 C for 4

hr, and bacterial growth was determined by measurement of the optical density at 600 nm (Fig. 1). The increase in optical density corresponding to 100% survival was determined in a control tube containing no staphylococcin. The reciprocal of the dilution yielding 50% increase was taken to be the activity in arbitrary units per milliliter (A.U./ml). Specific activities are represented as arbitrary units per milligram of protein (A.U./mg).

Protein assay. The protein was determined by the method of Lowry (29) with bovine serum albumin as reference.

Induction. *S. epidermidis* 1580 was inoculated and grown overnight in 2% proteose peptone no. 3 (Difco). The overnight culture was diluted to about 10^8 cells/ml with the same medium. The diluted culture was transferred to a New Brunswick gyratory shaker at 37 C. When the culture reached a concentration of 6×10^8 cells/ml, mitomycin C was added in a final concentration of 0.1, 0.5, or 1 μg per ml. After 30 min the cells were harvested by centrifugation and incubated in proteose peptone medium for an additional 4 hr.

Induction by ultraviolet irradiation was performed with an ultraviolet lamp (Mineralight R51) at a distance of 25 cm for 5, 10, 15, 20, and 30 sec (about 800 ergs per cm^2 per sec). A 10-ml bacterial suspension (10^8 cells/ml) in a 10-cm petri dish was used.

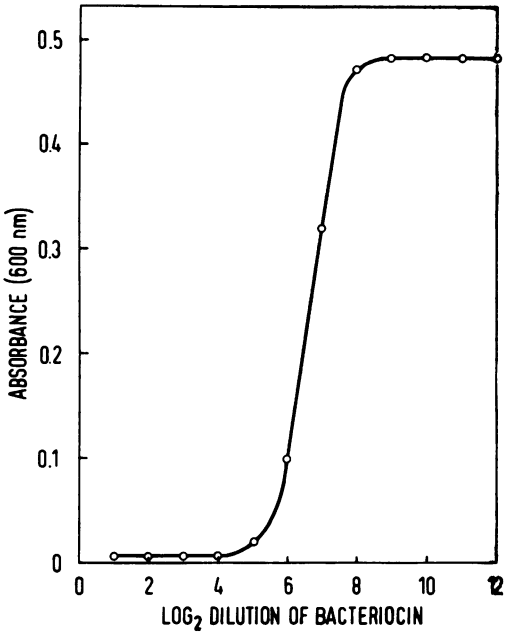

FIG. 1. *Quantitative determination of the staphylococcin activity. Serial dilutions (1 ml) of the bacteriocin were mixed with 1 ml of exponential-phase culture of the indicator strain (10^7 cells/ml). After incubation, bacterial growth was determined by measurement of the absorbance at 600 nm. The reciprocal of the dilution yielding 50% increase in absorbance with respect to the control (containing no staphylococcin) was taken to be the activity in arbitrary units per milliliter.*

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed according to the methods described by Ornstein (33) and Weber (39). Gels (3 and 10%) in 0.05 M phosphate buffer (*p*H 7.0) with or without 0.1% sodium dodecyl sulfate (SDS) were used; 5 mA was applied per tube (diameter 6 mm) at room temperature. The proteins were stained with Coomassie brillant blue R 250 (12), and the carbohydrates were located with the periodic acid-Schiff reaction (12). The staphylococcin activity and lipid compounds could be detected after removal of the SDS by soaking the gels in 0.01 M tris(hydroxymethyl)aminomethane - hydrochloride buffer (*p*H 7.0) for 18 hr. To detect lipid compounds the gels were stained with Oilred O (12). The staphylococcin activity could be located by embedding the gel in 1.3% agar plus 3% TSB and by spraying the indicator strain over it. After one night at 37 C the inhibition zone locates the staphylococcin 1580.

Continuous flow dialysis. Continuous flow dialysis was performed in an ultrafiltration cell model 52 from Amicon Co., Lexington, Mass.

Chemicals. Mitomycin C and Antifoam B were purchased from Sigma Chemical Co., St. Louis, Mo. Sephadex G-200 and G-50 were obtained from Pharmacia, Uppsala, Sweden. Trypticase soy broth was purchased from BBL, Cockeysville, Md. Acrylamide, *N,N,N',N'*-tetramethylethylenediamine and methylenebisacrylamide were purchased from Fluka A. G., Buchs, Switzerland. Proteose peptone no. 3, nutrient broth, and brain heart infusion were purchased from Difco Laboratories, Detroit, Mich.

RESULTS

Demonstration of bacteriocin production. *S. epidermidis* 1580 produced a substance with antagonistic activity against various staphylococci and other gram-positive bacteria. This was demonstrated first by using a technique described by Gratia (14). *S. epidermidis* 1580 was inoculated as a spot on a solid medium and incubated at 37 C for 18 hr. Then the indicator strain *S. aureus* Oxford 209 P was sprayed over it. After another 18-hr incubation an inhibition zone could be detected around the inoculum. This inhibition zone was not due to the production of acid or base or phages but to a bacteriocin called staphylococcin 1580 in this study. No phages had been produced since material extracted with water from the inhibition zone did not produce plaques when incubated at 37 C for 24 hr in soft brain heart infusion agar containing 10^6 cells of *S. aureus* Oxford 209 P.

Production of staphylococcin 1580. *S. epidermidis* 1580 produced the bacteriocin in well aerated liquid cultures in which foaming was restricted by Antifoam B (Sigma). No staphylococcin activity could be detected in the supernatant fluid or in the pellet obtained by

centrifugation of a *S. epidermidis* 1580 culture grown under anaerobic conditions. Addition of antifoam was necessary since this staphylococcin was very sensitive to mechanical agitation as was shown by a rapid inactivation upon bubbling air through an active preparation. This inactivation was not due to oxidation since mechanical agitation in a pure nitrogen atmosphere caused the same effect.

Various media, including proteose peptone no. 3, Trypticase soy broth (TSB), brain heart infusion, a synthetic medium as described by Richmond (36), and nutrient broth were compared for staphylococcin production (Table 1). The largest production and the highest specific activity were obtained in the TSB medium. No detectable amounts of the bacteriocin were produced in the synthetic medium. Activity was always found both in the supernatant fluid (about 80%) and the pellet (about 20%). The bacteriocin activity of all supernatant fractions was greatly enhanced by dialysis. Possibly, the cultures contain a small-molecular-weight inhibitor either produced by *S. epidermidis* 1580 or present in the original media. This inhibiting substance needs further study. The staphylococcin can be obtained from the bacterial pellet by extraction with 5% NaCl in 0.05 M phosphate buffer (*p*H 7.0). In a similar way colicins were previously isolated by Herschman and Helinski (17).

The optimal temperature and *p*H range for staphylococcin production were between 35 and 38 C and between *p*H 6.5 and 8.0, respectively.

TABLE 1. *Comparison of staphylococcin production in various media*[a]

Medium	Activity in supernatant		Activity in pellet (A.U.)	Specific activity	
	Before dialysis (A.U.)[c]	After dialysis (A.U.)		Supernatant[b] (A.U./mg)	Pellet (A.U./mg)
Proteose peptone no. 3	0	90	10	0.27	0.1
Brain heart infusion	0	180	40	0.51	0.2
Nutrient broth	0	110	20	0.62	0.15
Synthetic medium	0	0	0	0.0	0.0
Trypticase soy broth	50	400	80	1.0	0.45
Dialyzed Trypticase soy broth	50	400	80	93.0	0.45

[a] The pellet and supernatant of a 100-ml culture was tested.

[b] Determined after dialysis.

[c] A.U., Arbitrary units; see Materials and Methods.

The amount of staphylococcin 1580 present in liquid cultures was too small to allow an efficient purification procedure. Moreover, staphylococcin production was not induced by ultraviolet irradiation or treatment with mitomycin C (Fig 2). In the presence of 0.5 μg of mitomycin C per ml or with ultraviolet irradiation of 15 sec the highest phage titer of about 10^6 plaque-forming units per ml was measured. Upon growth of the cells on a semisolid medium, a crude staphylococcin preparation was obtained with an activity 20 times higher than that in liquid cultures of the same volume.

Extraction of staphylococcin 1580. *S. epidermidis* 1580 was grown in petri dishes containing a semisolid medium consisting of 0.4% agar and dialyzed TSB at 37 C for 48 hr. Dialyzed TSB was the outer fluid obtained on dialysis of 60 g of TSB in 100 ml of water against 2 liters of water at 4 C for one night. A semisolid medium on the basis of dialyzed TSB was chosen to facilitate the further purification steps by the absence of high-molecular-weight substances. After the incubation

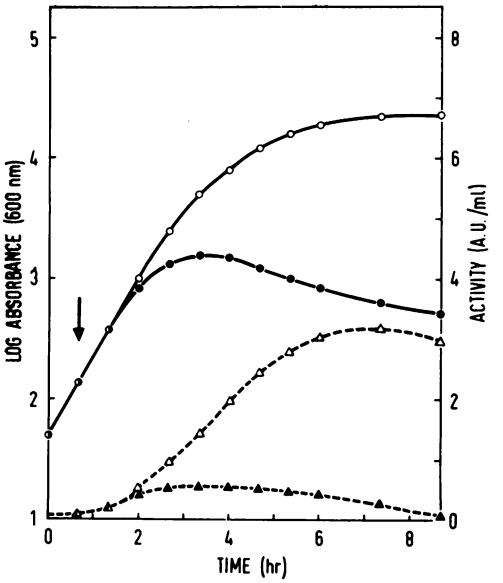

FIG. 2. *The effect of mitomycin C on the production of staphylococcin 1580. To a culture of Staphylococcus epidermidis 1580 in the exponential phase, mitomycin C (0.5 μg/ml) was added at the moment indicated by the arrow. Samples withdrawn at the times indicated were assayed for staphylococcin activity (▲) and absorbance at 600 nm (●). Samples of an untreated culture were assayed in the same way (open symbols).*

period the petri dishes were placed at −20 C for 4 hr. Thereafter, the medium was thawed. The contents of the petri dishes were pooled and the resulting slurry was centrifuged at 18,000 × *g* for 10 min in a Sorvall RC-2B centrifuge. The supernatant fluid obtained was designated as the crude staphylococcin preparation.

Purification of staphylococcin 1580. A scheme of the followed purification procedure is given in Fig. 3. A saturated solution of ammonium sulfate (*p*H 7.0) was added slowly to the crude preparation of staphylococcin under constant stirring at 4 C. The majority of the compound was obtained in the fraction precipitated between 0 and 55% saturation (Fig. 4). The 55% saturated suspension was further stirred for 30 min and centrifuged at 18,000 × *g* for 10 min. The pellet was dissolved in 0.05 M phosphate buffer (*p*H 7.0), and the clear solution was dialyzed for 18 hr against the same buffer. During dialysis an inactive precipitate was formed which was removed by centrifugation at 18,000 × *g* for 10 min. This procedure yielded a high purification (Table 2), because all the low-molecular-weight substances had been removed.

The next steps of the purification procedure are based on the high molecular weight of the staphylococcin and the dissociation of it into subunits in the presence of 6 M urea. The bacteriocin preparation was centrifuged at 150,000 × *g* in a Spinco L-50 ultracentrifuge for one night. The pellet containing all the activity was dissolved in 0.05 M phosphate buffer (*p*H 7.0). Upon centrifugation in 0.1% SDS or in 6 M urea the activity could be detected only in the supernatant fraction. After removal of the SDS or urea by dialysis and centrifugation of the staphylococcin preparation at 150,000 × *g*,

FIG. 3. *Isolation and purification of staphylococcin 1580.*

the activity could be detected again only in the pellet. Therefore, it is likely that the staphylococcin consists of subunits. The dissolved pellet was applied to a Sephadex G-200 column (2.8 by 44 cm) prepared in 0.05 M phosphate buffer (*p*H 7.0). The activity was eluted from the column in the first peak at the void volume (Fig. 5). The active fractions were pooled and concentrated in an ultrafiltration cell model 52 (Amicon). This preparation was applied to a Sephadex G-50 column (2.8 by 40 cm) prepared in 0.2 M phosphate buffer (*p*H 7.0) containing 6 M urea (Fig. 6). Now, the activity was eluted from the column in the second peak. The active fractions were pooled, dialyzed in a continuous-flow dialyzer model 52 (Amicon), and lyophilized. This substance is designated as the purified staphylococcin 1580. Lyophilization of purified staphylococcin 1580 resulted in a total loss of the activity which could be prevented by addition of 0.5% bovine serum albumin to the pure bacteriocin.

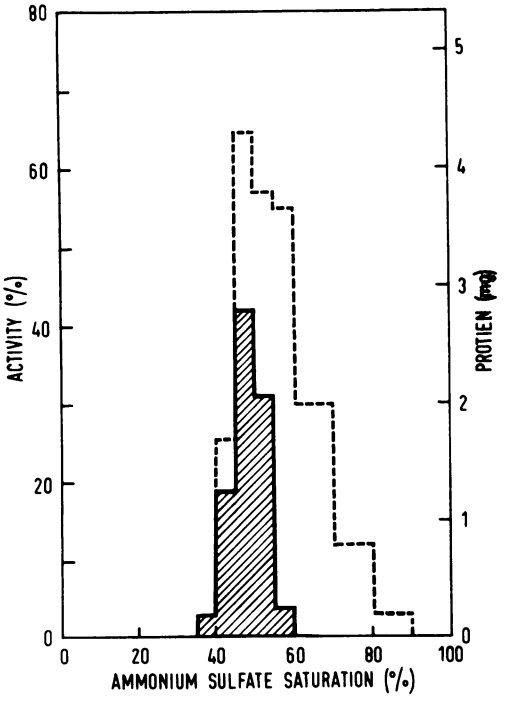

FIG. 4. *Distribution of the staphylococcin activity and protein in the fractions precipitated by ammonium sulfate. A crude staphylococcin preparation (100 ml) was fractionated by addition of a saturated ammonium sulfate solution. The precipitates formed were dissolved in 0.02 M phosphate buffer (pH 7.0) and after dialyzation the total staphylococcin activity (solid line) and protein (dashed line) was determined.*

The results of this purification are summarized in Table 2. Several other attempts were made to purify the staphylococcin further. Gradient elution from diethylaminoethyl-cellulose columns with a variety of sodium chloride and *p*H gradients, differential adsorption, and elution from calcium phosphate gels failed to yield preparations with increased specific activities. Upon precipitation with alcohol or acetone, a precipitate was formed which contained no activity. Staphylococcin 1580 was applied to a Sepharose 4B column (2.0 by 26 cm) prepared in 0.05 M phosphate buffer (*p*H 7.0). The staphylococcin was eluted as a single peak in the region of the low-molecular-weight substances. This means that the molecular weight of staphylococcin 1580 was smaller than 400,000.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis at *p*H 7.0 or 8.9 in the presence or absence of 0.1% SDS was used as a criterion of homogeneity of the purified staphylococcin 1580. In the absence of SDS it appeared as a large diffuse zone on 3% gels upon staining for protein, lipid, or carbohydrate, whereas in 10% gels the bacteriocin did not migrate from the origin and the activity could be detected at the top of the gel. In the presence of 0.1% SDS, dissociation into subunits occurred and the staphylococcin appeared as a single band coincident with the

TABLE 2. *Purification of staphylococcin 1580*[a]

Purification step	Vol (ml)	Arbitrary units (A.U.)	Protein (mg)	Specific activity (A.U./mg)	Recovery (%)	Times purified
Crude preparation	1,200	100,000[b]	5,250	19	100	1
Ammonium sulfate precipitate	60	95,000	12.0	7,900	95	415
150,000 × *g* pellet	10	95,000	4.0	23,800	95	1,250
Sephadex G-200 gel filtration	25	90,000	2.2	40,800	90	2,150
Sephadex G-50 gel filtration	50	82,000	0.73	112,500	82	5,900

[a] Eighty petri dishes (1,600 ml) were used.
[b] Determined after dialysis of the crude preparation.

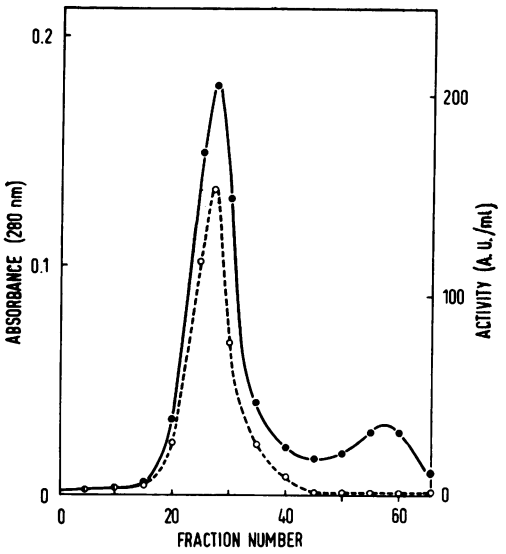

FIG. 5. *Sephadex G-200 gel filtration of staphylococcin 1580. The elution occurred in 0.05 M phosphate buffer (pH 7.0). Each 5-ml fraction was assayed for staphylococcin activity (○) and protein (●). Tubes 21 through 34 were pooled yielding a 95% recovery of the total activity applied to the column.*

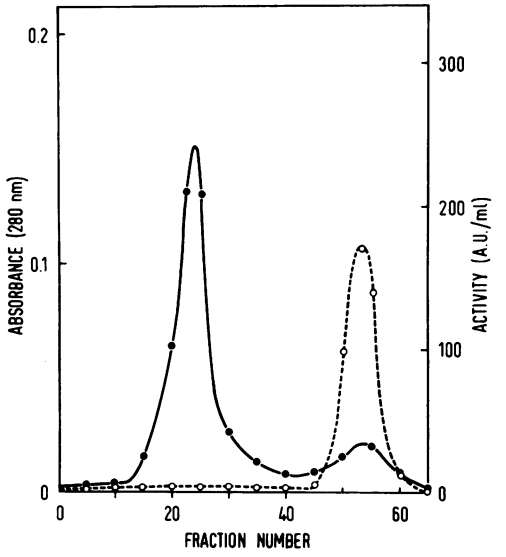

FIG. 6. *Sephadex G-50 gel filtration of staphylococcin 1580. The elution occurred with 0.2 M phosphate buffer (pH 7.0) containing 6 M urea. Each 5-ml fraction was assayed for staphylococcin activity (○) and protein (●). The tubes 45 through 57 were pooled yielding a 90% recovery of the total activity applied to the column.*

zone which stained for protein, lipid, and carbohydrate (Fig. 7). Comparison with the position of molecules with known molecular weight showed that the subunit must have a molecular weight between 10,000 and 25,000. Controls were run in order to detect interference of SDS in the determination of the lipid and bacteriocin activity band.

DISCUSSION

Bacteria grown on semisolid Trypticase soy agar yielded the largest extracellular staphylococcin production and the highest specific activity. The production was optimal under the same conditions which are optimal for the production of extracellular proteins (37). In contrast to the staphylococcins isolated by Dajani et al. (5) and Gagliano and Hinsdill (10), staphylococcin 1580 could be obtained from the cells by extraction with 5% NaCl solution. Colicin E2 and colicin E3 can also be obtained by extraction with a NaCl solution (17). In contrast to many other bacteriocins, the staphylococcin 1580 production could not be in-

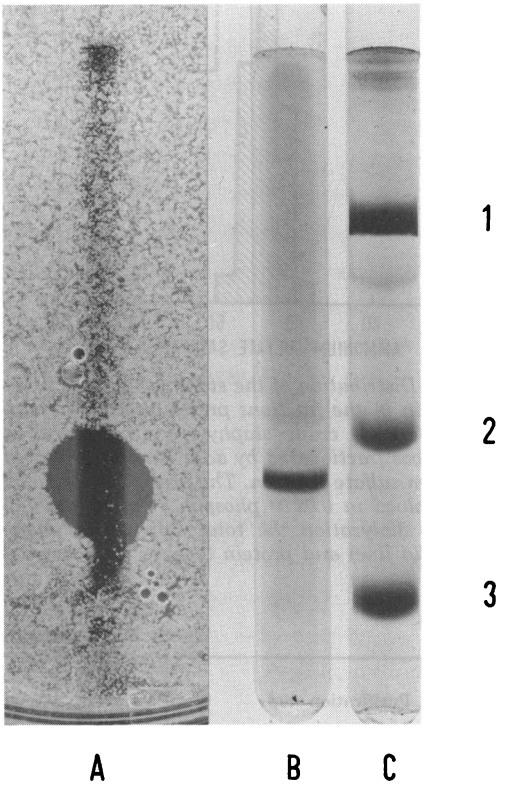

FIG. 7. *Polyacrylamide gel electrophoresis of purified staphylococcin 1580 on 10% gels with 0.1% sodium dodecyl sulfate. The gels were tested on staphylococcin activity (gel A) and stained for protein (gel B). Serum albumin (1), α-chymotrypsinogen (2), and cytochrome C (3) were used as references (gel C).*

duced by ultraviolet irradiation or treatment with mitomycin C. This is in agreement with the results of Gagliano and Hinsdill (10) and Dajani et al. (4).

Purification of staphylococcin 1580 was achieved by a combination of ammonium sulfate precipitation, dialysis, ultracentrifugation, and gel filtration on Sephadex columns.

Many bacteriocins have been purified in recent years. Bacteriocins from certain *Escherichia coli* strains (30) as well as from *Pseudomonas aeruginosa* (18), *Listeria monocytogenes* (15), *Vibrio comma* (40), and *Lactobacillus acidophilus* (25) resemble elements of bacteriophages or whole phages. Megacin C, a bacteriocin produced by *Bacillus megaterium* (20), colicin E2 and E3 (17), and cloacin DF 13, a bacteriocin produced by *Enterobacter cloacae* DF 13 (13), are simple proteins with molecular weights of approximately 60,000.

Different from these bacteriocins are those which have been identified as lipo-polysaccharide-protein complexes. Colicin V-K 357 (22) and colicin I, produced by *Salmonella strasbourg* (24), and a bacteriocin produced by *Lactobacillus fermenti* strain 466 (26) have been shown to be lipo-polysaccharide-protein complexes. Staphylococcin 1580 seems to belong to this class of bacteriocins, whereas the bacteriocin produced by *Staphylococcus aureus* type 71, isolated by Dajani et al. (5), is only proteinaceous of nature. Staphylococcin 1580 resembles the bacteriocin isolated by Gagliano and Hinsdill (10) as to the chemical composition but differs as to the behavior during production and isolation. Staphylococcin 1580 is totally different from the staphylococcin recently isolated by Dobardzic et al. (8) which appeared to be a phage-like particle. Comparison with other staphylococcins is not possible because the lack of data (2, 11, 21, 28).

Urea and SDS split staphylococcin 1580 into smaller units, which migrated as one band on polyacrylamide gel electrophoresis and consisted of protein, lipid, and carbohydrate. The dissociation and association of the staphylococcin into subunits was a reversible process. Gel filtration indicated that the molecular weight of the subunits is between 10,000 and 25,000. However, this molecular weight cannot be calculated exactly from the position on the polyacrylamide gel with respect to the position of the references (39), since the subunits consist of protein, lipid, and carbohydrate and the references consist only of protein. Protein generally exposes a much more compact structure than carbohydrate.

A subsequent paper deals with a further study of the chemical nature and some properties of staphylococcin 1580.

LITERATURE CITED

1. Barrow, G. 1963. Microbial antagonism by *Staphylococcus aureus*. J. Gen. Microbiol. **31**:471-481.
2. Barrow, G. 1963. The nature of inhibitory activity by *Staphylococcus aureus* type 71. J. Gen. Microbiol. **32**: 255-261.
3. Bazaral, M., and D. Helinski. 1968. Circular DNA forms of colicinogenic factors E1, E2 and E3 from *Escherichia coli*. J. Mol. Biol. **36**:185-194.
4. Dajani, A., E. Gray, and L. Wannamaker. 1970. Bactericidal substance from *Staphylococcus aureus*. J. Exp. Med. **131**:1004-1015.
5. Dajani, A., and L. Wannamaker. 1969. Demonstration of a bactericidal substance against β-hemolytic streptococci in supernatant fluids of staphylococcal cultures. J. Bacteriol. **97**:985-991.
6. Dajani, A., E. Gray, and L. Wannamaker. 1970. Effect of a bactericidal substance from *Staphylococcus aureus* on group A streptococci. Infect. Immunity **1**:485-490.
7. Dandeu, J. 1971. Chemical and immunological study of colicins E1, K, A, and Q. Infect. Immunity **3**:1-9.
8. Dobardzic, R., P. Payment, and S. Sonea. 1971. Ultrastructure du bactériophage ϕ RE agissant dans certaines conditions comme une staphylococcine. Can. J. Microbiol. **17**:847-849.
9. Frederique, P. 1946. Sur la sensibilité et l'activité antibiotique des staphylococciques. C. R. Soc. Biol. **140**: 1167-1170.
10. Gagliano, V., and R. Hinsdill. 1970. Characterization of a *Staphylococcus aureus* bacteriocin. J. Bacteriol. **104**: 117-125.
11. Gardner, J. 1949. An antibiotic produced by *Staphylococcus aureus*. Brit. J. Exp. Pathol. **30**:130-138.
12. Gordon, A. 1969. Electrophoresis of proteins in polyacrylamide and starch gels. North Holland Publishing Co., Amsterdam.
13. Graaf, F. de, L. Goedvolk-de Groot, and A. Stouthamer. 1970. Purification of a bacteriocin produced by *Enterobacter cloacae* DF 13. Biochim. Biophys. Acta **221**: 566-575.
14. Gratia, A. 1946. Techniques sélectives pour la recherche systématique des germes antibiotiques. C. R. Soc. Biol. **140**:1053-1056.
15. Hamon, Y. 1966. Sur la nature des bacteriocines produits par Listeria monocytogenes. C. R. Acad. Sci. Ser. D. **263**:198-201.
16. Hamon, Y., and Y. Peron. 1963. Quelques remarques sur les bacteriocines produites par les microbes grampositives. C. R. Acad. Sci. Ser. D. **257**:1191-1193.
17. Herschman, H., and D. Helinski. 1967. Purification and characterization of colicin E2 and colicin E3. J. Biol. Chem. **242**:5360-5368.
18. Higerd, T., C. Baechler, and R. Berk. 1969. Morphological studies on relaxed and contracted forms of purified pyocin particles. J. Bacteriol. **98**:1378-1389.
19. Hill, C., and I. Holland. 1967. Genetic basis of colicin E susceptibility in *Escherichia coli*. J. Bacteriol. **94**:677-686.
20. Holland, I. 1961. The purification and properties of megacin, a bacteriocin from *Bacillus megaterium*. Biochem. J. **78**:641-648.
21. Hsu, C., and G. Wiseman. 1967. Antibacterial substances from staphylococci. Can. J. Microbiol. **13**:947-955.
22. Hutton, J., and W. Goebel. 1961. Colicin V. Proc. Nat. Acad. Sci. U.S.A. **47**:1498-1500.
23. Ivanovics, G. 1962. Bacteriocins and bacteriocin-like substances. Bacteriol. Rev. **26**:108-118.

24. Keene, J. 1966. Preparation and chemical properties of colicin I. Can. J. Microbiol. **12**:425–427.
25. Klerk, H. de, and N. Hugo. 1970. Phage-like structures from *Lactobacillus acidophilus*. J. Gen. Virol. **8**:231–234.
26. Klerk, H. de, and J. Smit. 1967. Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. **48**:309–316.
27. Lachowicz, T. 1965. Investigations on staphylococcins. Zentralbl. Bakteriol. Parasitenk. Abt. I. Orig. **196**:340–351.
28. Lachowicz, T., and Z. Walczak. 1968. Purification and properties of staphylococcin A. Arch. Immunol. Ther. Exp. **16**:855–863.
29. Lowry, O., N. Rosebrough, A. Farr, and R. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
30. Mennigman, H. 1965. Electronmicroscopy of the antibacterial agent produced by *Escherichia coli 15*. J. Gen. Microbiol. **41**:151–154.
31. Nomura, M. 1964. Mechanism of action of colicins. Proc. Nat. Acad. Sci. U.S.A. **52**:1514–1521.
32. Nomura, M. 1967. Colicins and related bacteriocins. Annu. Rev. Microbiol. **21**:257–280.
33. Ornstein, L. 1964. Disc electrophoresis. Ann. N.Y. Acad. Sci. **121**:321–427.
34. Parker, M., and L. Simmons. 1959. The inhibition of *Corynebacterium diphteriae* and other gram-positive organisms by *Staphylococcus aureus*. J. Gen. Microbiol. **21**:457–476.
35. Reeves, P. 1965. The bacteriocins. Bacteriol. Rev. **29**:24–45.
36. Richmond, M. 1959. The differential effect of arginine and canavacine on growth and enzyme formation in *Staphylococcus aureus* 524 SC. Biochem. J. **73**:155–166.
37. Rogers, H. 1954. The rate of formation of hyaluronidase coagulase and total extracellular protein by strains of *Staphylococcus aureus*. J. Gen. Microbiol. **10**:109–121.
38. Tieze, G., and A. Stouthamer. 1969. A bacteriocinogenic factor of *Enterobacter cloacae*. Mol. Gen. Genet. **106**: 48–65.
39. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. **244**: 4406–4412.
40. Yayawardene, A., and H. Farkas-Himsley. 1969. Vibriocin: a bacteriocin from *Vibrio comma* I. Production, purification, morphology and immunological studies. Microbios **1B**:87–98.