ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1975, p. 139-145 Vol. 7, No. 2
Copyright © 1975 American Society for Microbiology
Printed in U.S.A.

# Production and Mode of Action of Lactocin 27: Bacteriocin from a Homofermentative *Lactobacillus*

G. C. UPRETI AND R. D. HINSDILL*

*Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706*

Received for publication 9 October 1974

*Lactobacillus helveticus* strain LP27 produced a bacteriocin, lactocin 27, in dialyzable and nondialyzable forms. No evidence was obtained to indicate that lactocin 27 was under the control of extrachromosomal plasmids. Lactocin 27 had a bacteriostatic effect on the indicator, *Lactobacillus helveticus* strain LS18. It inhibited primarily protein synthesis without affecting deoxyribonucleic acid and ribonucleic acid synthesis or adenosine 5'-triphosphate levels. Treatment of susceptible cells with the lactocin did not cause leakage of ultraviolet-absorbing material, but caused the efflux of potassium ions and the influx of sodium ions. It adsorbed non-specifically to various bacterial species irrespective of their susceptibility to lactocin 27. However, the presence of specific receptors has not been ruled out.

Bacteriocins are antibiotic-like proteinaceous substances synthesized by certain strains of bacteria and are active against closely related species. The colicins produced by *Escherichia coli* and other species of *Enterobacteriaceae* have been studied extensively (15, 17). The recent studies with colicins ($E_1$, $E_2$, $E_3$, and K) suggest that the colicins manifest their biochemical effect(s) by interacting with their target directly (1, 2, 3, 4, 16, 18). The specificity of colicins for *E. coli* and other closely related species, however, lies at the level of the receptor (19). Similar in vitro studies have not been conducted for the bacteriocins from gram-positive bacteria.

Little is known about the bacteriocins of lactobacilli. DeKlerk and Smit (8) characterized a bacteriocin from a heterofermentative *Lactobacillus fermenti*, but the mode of action was not studied. We previously characterized lactocin 27 from a homofermentative *Lactobacillus helveticus*, strain LP27 (20). The present paper describes aspects of production and the mode of action of lactocin 27.

## MATERIALS AND METHODS

**Media, bacterial strains, isolation, and purification of lactocin.** Isolation of lactocin 27, assay of its activity in arbitrary units, and lactocinogenic- and lactocin-susceptible strains have been described in a previous paper (20). In the experiments described here, partially purified lactocin (referred to as lactocin 27) was used rather than purified lactocin. The latter had an undetermined amount of bound sodium dodecyl sulphate (SDS). Portions of lactocin 27 in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.7), containing 1 mg of protein per ml, as determined by the Lowry method (14), were stored at −20 C. Once thawed, the solution could be stored in the refrigerator and used over several weeks without a significant drop in activity. The various bacterial suspensions used in this work were made up in sterile Ringer solution prepared by first dissolving 2.15 g of NaCl, 0.075 g of KCl, 0.12 g of $CaCl_2$, and 0.5 g of $Na_2S_2O_3 \cdot 5H_2O$ per liter of distilled water, then diluting fourfold before use.

**Permeation of lactocin.** A section of thin-wall dialysis tubing (Union Carbide, Chicago, Ill.) was cut open to give a flat piece of dialysis membrane (3 by 10 cm), washed with distilled water, and sterilized by autoclaving. The dialysis membrane was placed aseptically on an APT (Difco, Detroit, Mich.) agar plate containing 0.01% sodium azide. The azide is not essential but greatly reduces the chances of mold contamination. Two sterilized cylinders (inner diameter 2 cm) were placed 3 to 4 cm apart over the dialysis membrane. Lactocin 27 (0.1 ml) and an exponentially growing culture of *L. helveticus* strain LP27 (0.1 ml) were mixed separately with 3 ml of APT soft agar (APT broth containing 0.75% agar). Two drops of soft agar containing lactocin 27 and *L. helveticus* strain LP27 cells, respectively, were placed inside the hollow cylinders over the dialysis membrane and also on the portion of the ATP agar plate not covered by dialysis membrane (see Fig. 1). The plate was incubated at 37 C for 24 h. The hollow cylinders and dialysis membranes were aseptically removed. The plate was then exposed to chloroform and overlayed with susceptible *L. helveticus*, strain LS18, as described earlier (20) to detect the presence of dialyzable inhibitory substance. The specificity of the inhibitory substance was confirmed by repeating the experiment, but using resistant *L. helveticus*, strain LP27, and another known resistant isolate of homofermentative lactobacilli (species not identified). The possible destruction of dialysis membrane by growing cells of *L. helveticus* strain LP27 was tested for by reusing the dialysis membrane and

spotting lactocin 27 in place of *L. helveticus* strain LP27 cells.

**Curing of lactocin production.** Attempts were made to cure lactocin production by the methods previously described (5) with minor modifications. *L. helveticus* strain LP27 was grown in APT broth in 10-ml portions in the presence of various concentrations of the following potential curing agents for 18 h: rifampin (0.01 to 0.075 $\mu$g/ml), acridine orange (2 to 10 $\mu$g/ml), neutral acriflavin (2 to 20 $\mu$g/ml), and SDS (100 to 750 $\mu$g/ml). The final concentration in each case was inhibitory to growth. For possible curing at higher temperature, the LP27 culture was grown at 42 C. A loopful of cell suspension from the above treatments was streaked on APT agar plates, which were later incubated at 37 C for 36 h. Single colonies (150 to 200) were picked up and stabbed on APT agar plates (seven colonies per plate) and tested for lactocin production. Evidence of spontaneous loss of lactocin production was sought by testing 400-single-colony isolates from the untreated culture.

**Assay of lactocin activity.** Lactocin activity could be expressed either in arbitrary units (20) or in inhibitory units (I.U.), which was determined as follows: *L. helveticus* strain LS18 was grown to mid-exponential phase (0.4 absorbancy units at 615 nm). One milliliter of this culture was transferred to 4 ml of APT broth and incubated at 37 C for 40 min (zero time = $T_0$), which corresponds to the end of the lag phase. At $T_0$, 0.1 ml of an appropriate dilution of lactocin 27 stock solution was added to the 5 ml of culture and further incubated for 2 h. The concentration which resulted in a 50% inhibition in culture absorbancy (615 nm) was defined as 1 I.U. For this particular culture, this point also corresponds very closely to a 50% inhibition in colony-forming units (CFU).

**Effect of lactocin on biosynthesis of macromolecules.** Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis in lactocin 27-susceptible *L. helveticus*, strain LS18, were measured by the incorporation of radioactive precursors into trichloroacetic acid-precipitable material. Appropriate amounts of [$^3$H]thymidine (0.5 $\mu$Ci/ml), [$^3$H]uridine (0.5 $\mu$Ci/ml), and [$^3$H]isoleucine (1.0 $\mu$Ci/ml) were added to LS18 culture at *T*-5 min. The desired amount of lactocin 27 (buffer in case of control) was added at $T_0$. The effects of lactocin 27 were compared with known inhibitors. At various time intervals, duplicate 1-ml samples of the culture were precipitated with cold 10% trichloroacetic acid (final concentration 5% wt/vol). In the case of [$^3$H]isoleucine incorporation, the samples were kept in boiling water for 20 min to remove labeled isoleucine from transfer RNA-isoleucine complex. The insoluble material was collected over glass fiber filters (GF/C Whatman, England) and washed with approximately 15 ml of cold 5% trichloroacetic acid. The filters were dried by exposure to infrared light for 20 min and then transferred to a vial to which 5 ml of scintillation fluid was added. The scintillation fluid consisted of 3 g of 2,5-diphenyloxazole plus 100 mg of 1,4 bis-2-(4-methyl-5 Phenyloxazolyl)-benzene dissolved in 1 liter of toluene. The samples were counted in a Packard scintillation counter model 3775 after allowing 1 h for cold equilibration. Tritiated thymidine and uridine were supplied by New England Nuclear (Boston, Mass.) and isoleucine was supplied by Amersham/Searle (Arlington Heights, Ill.)

The specific incorporation of [$^3$H]thymidine into DNA and [$^3$H]uridine into RNA was checked by dividing the culture into three portions. The first portion was treated with chilled trichloroacetic acid (final concentration 5% wt/vol) previously described to act as the control. The second (acid hydrolyzed) portion was treated with trichloroacetic acid (10% wt/vol final concentration) but heated to approximately 100 C for 30 min. The sample was then chilled and diluted with water to lower the trichloroacetic acid concentration to 5% before filtration. The third (alkali hydrolyzed) portion was first treated with NaOH (1 N final concentration) for 6 h at 37 C. An equal volume of 1 N HCl was added to neutralize excess NaOH and the sample was chilled. Cold trichloroacetic acid was added to give a final concentration of 5% before filtration.

**Measurement of intracellular adenosine 5′-triphosphate (ATP).** Cells were treated with 8 I.U. of lactocin 27 (buffer in case of control) at $T_0$. The ATP levels of the treated and control cells were determined as follows. Samples (0.1 ml) were removed at intervals and extracted with 0.9 ml of 90% dimethyl sulfoxide (Mallinckrodt, St. Louis, Mo.) for 2 min. To the extracted sample, 5 ml of morpholinopropane-NaOH buffer (0.1 M, pH 7.4) was added; the sample was then stored at −20 C until used. The preparations were assayed using an ATP-Biometer (model 760, DuPont Instruments, Wilmington, Del.) and a buffer-salt tablet and enzyme substrate kit (DuPont). The instrument was calibrated with a standard solution of ATP (Sigma Standard, Sigma Chemical Co., St. Louis, Mo.).

**Effect of lactocin on cell membrane.** Leakage of ultraviolet (UV) light-absorbing material and transport of various ions were used as indicators of cell membrane integrity.

At time $T_0$, the LS18 cells were collected on filters (pore size 0.45 $\mu$m, Millipore Corp., Bedford, Mass.) and washed with Ringer solution. The cells were then resuspended in Ringer solution to the original volume and treated with 8 I.U. of lactocin 27 or buffer (control) at 37 C. Samples at various time intervals were filtered and a portion of the 60-min sample was plated to determine CFU. The absorbance of extracellular fluid was measured at 260 and 280 nm.

The cobalt, magnesium, manganese, sodium, and potassium contents of control and treated cells were measured by neutron activation analysis as follows. The *L. helveticus* strain LS18 cells were treated in APT broth with 8 I.U. of lactocin 27 or buffer (control) at time $T_0$. Samples (10 ml) were taken periodically, and filtered immediately using a membrane filter (0.45-$\mu$m pore size, Millipore Corp.). The cells were washed with ice cold isotonic 0.25 M sucrose solution. The cells were then suspended in 5 ml of doubly distilled water, a 1-ml sample was withdrawn for dry

weight determinations, and 4 ml of 2 M $H_2SO_4$ was added to the rest of the cell suspension. The cells in $H_2SO_4$ were extracted by heating for 15 min at 100 C. After cooling, the hydrolysate was clarified by centrifugation (8000 × *g* for 20 min) and a 5-ml sample was sealed in a pneumatic tube (Wisconsin nuclear reactor). The samples were irradiated by neutron activation for 5 min in the estimation of $Co^{2+}$, $Mn^{2+}$, and $Na^+$ and for 10 min in the case of $K^+$ and $Mg^{2+}$. After waiting 2 min, the samples were counted 10 min for $Co^{2+}$, $Mn^{2+}$, and $Na^+$ and 15 min for $K^+$ and $Mg^{2+}$ in a Lithium Drifted Germanium detector (Nuclear Diodes, Prairie View, Ill.). In each case, atomic adsorption standards of Fisher Chemical Co. (Fair Lawn, N.J.) were used. The pneumatic tubes were not handled by bare hands and plastic ware was used to prepare all samples.

**Adsorption of lactocin 27.** For adsorption studies *L. helveticus* strain LS18 (susceptible strain) and the following gram-positive organisms, which are resistant to lactocin 27, were used: *Bacillus cereus*, *B. megaterium*, *B. polymyxa*, *Micrococcus flavus*, *M. freudenreichii*, *Sarcina lutea*, *Streptococcus lactis*, *S. faecium*, *S. equisimilis*, *Staphylococcus aureus*, and *L. helveticus* strain LP27 (lactocinogenic strain). In addition, gram-negative *E. coli* was used. Except for lactobacilli, the microorganisms were grown on nutrient agar plates at 37 C overnight. The cells were harvested, washed, and resuspended in Ringer solution (0.5 absorbancy units at 415 nm). *L. helveticus* LS18 was used to determine the maximum concentration of lactocin 27 which could be used in the adsorption experiments and still get complete adsorption, i.e., lack of any detectable bacteriocin activity in the culture filtrate. The same concentration of lactocin 27 was used for all other cultures, allowing the adsorption to proceed for 15 min at 37 C. The suspension was then filtered (Millipore Corp., 0.45-μm pore size) and lactocin activity of the filtrate was determined in arbitrary units as described previously (20). A solution of lactocin, without the bacteria, was heated, filtered, and assayed again as a control.

## RESULTS

**Diffusibility of lactocin.** Lactocin, when isolated from the culture supernatant, was a nondialyzable, large-molecular-weight lipoprotein-carbohydrate complex, which could subsequently be dissociated by SDS into small-molecular-weight entities. The active moiety is believed to be a glycoprotein, molecular weight 12,400 (20). When *L. helveticus* strain LP27 was grown over the thin-wall dialysis membrane, the inhibitory activity could diffuse readily into the agar below without destroying the dialysis membrane (Fig. 1).

**Effect of potential curing agent on lactocin production.** The spontaneous loss of lactocin production from a freshly transferred culture of *L. helveticus* strain LP27 was less than 1%. We did note, however, that storage of dilute broth cultures at −20 C for approximately 1 year decreased the proportion of CFU able to produce bacteriocin by approximately 50%. For this experiment, the frozen cultures were thawed and incubated for 18 h at 37 C. A loopful of broth was streaked on APT agar and, after incubation for 36 h, individual colonies were tested for lactocin production as previously described.

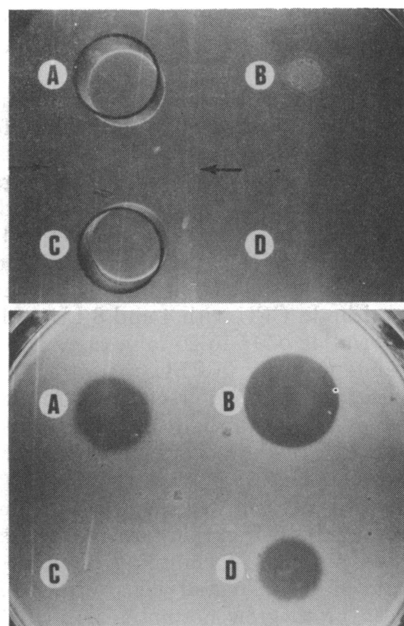


Fig. 1. *Permeability of the bacteriocin from L. helveticus LP 27. (Top) Cells of strain LP27 were placed on spots A and B, and lactocin 27 on spots C and D. The arrows indicate the margins of the thin dialysis membrane. (Bottom) Growth of susceptible L. helveticus strain LS18 on the above plate after removal of the cylinders and dialysis membrane and seeding of the plate. Dialyzable lactocin as shown on spot A (clear zone) and nondialyzable lactocin on spot C (absence of clear zone). Spots B and D show the inhibitory activity of L. helveticus strain LP27 cells and isolated lactocin 27.*

*L. helveticus* strain LP27 was grown in the presence of SDS, rifampin, ethidium bromide, acridine orange, and neutral acriflavin and also at higher temperature (42 C). Every isolated colony tested in this fashion retained its ability to produce lactocin. Thus, the lactocin production could not be cured by these treatments.

**Inhibitory effect of lactocin 27.** *L. helveticus* strain LS18 overlayed on a spot of lactocin 27 on a APT agar plate showed a clear zone upon overnight incubation. The zone of clearance used to express lactocin 27 activity in terms of arbi-

trary units (20) does not distinguish between possible bacteriostatic and bactericidal actions of lactocin 27.

The absorbancy (615 nm) of a broth culture of *L. helveticus* strain LS18 treated with different concentrations of lactocin 27 for 2 h showed an inverse relationship with lactocin concentration (Fig. 2). The concentration which resulted in a 50% inhibition in absorbance was defined as 1 I.U. as previously explained. The growth curve in the presence of lactocin 27 (Fig. 3) showed a sharp drop in CFU with 4 and 8 I.U. of lactocin. After a lag of 15 to 20 min, there appeared to be some recovery in CFU and, after 60 min, the counts maintained a constant level. The absorbancy is substantially less than the control, although there is some increase even with 8 I.U. of lactocin 27. Microscopically, the cells treated with lactocin (8 I.U.) appeared normal and retained their gram-positive character. There was no sign of clumping of either cells or short chains. Treated cells when diluted into fresh broth grew normally, i.e., CFU and optical density increased at the normal rate, and a second exposure of these cells to 8 I.U. of lactocin 27 resulted in an inhibitory curve similar to Fig. 3 (data not shown).

**Effect of lactocin 27 on macromolecular synthesis.** Treatment of *L. helveticus* strain LS18 cells with lactocin 27 resulted in inhibition of the incorporation of [$^{3}$H]isoleucine (Fig. 4).

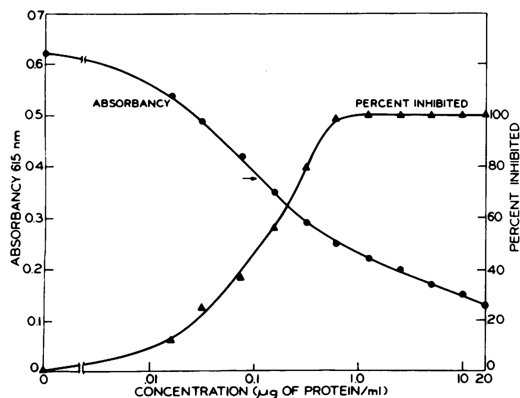


FIG. 2. *Effect of different concentrations of lactocin 27 on L. helveticus strain LS18. An inverse relationship was observed between lactocin concentration and absorbance. A 50% inhibition in absorbance was defined as 1 I.U. For this organism this amounts to half the difference between the initial (0.13) and final (0.62) absorbance of the control culture without lactocin, i.e., 0.375 (arrow), which is achieved by the addition of approximately 0.15 µg of protein/ml to the treated culture. For this particular culture this point also corresponds to roughly 50% inhibition of CFU.*

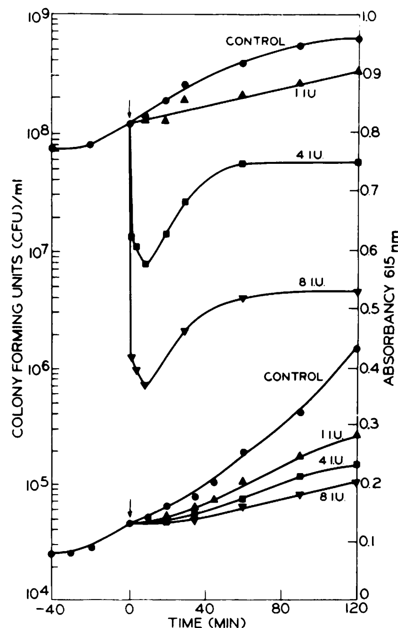


FIG. 3. *Effects of 1, 4, and 8 I.U. of lactocin 27 on growth and colony-forming ability of L. helveticus strain LS18. Lactocin and buffer (in case of control) were added at $T_0$ (indicated by arrows). Upper series of curves represent CFU, bottom series represent absorbancy (615 nm).*

There was no immediate effect on the incorporation of [$^{3}$H]thymidine or [$^{3}$H]uridine, but some decrease was noted after 30 to 40 min (Fig. 5 and 6). The effect at later stages seems secondary to inhibition of protein synthesis. The nonspecific incorporation of [$^{3}$H]thymidine and [$^{3}$H]uridine in macromolecules other than DNA and RNA was ruled out by comparing the counts of trichloroacetic acid-insoluble material before and after alkali and acid hydrolysis (Table 1 and 2).

**ATP level.** The ATP level was determined as previously described. The ATP content of control and lactocin 27-treated cells of *L. helveticus* strain LS18 did not differ significantly over a 60-min period.

**Effect of lactocin 27 on cell membrane permeability.** The effect of lactocin on the integrity of cell membrane was measured using two parameters. Treatment of *L. helveticus* strain LS18 cells with lactocin did not cause leakage of UV-absorbing material measured at 260 and 280 nm.

The sodium, potassium, cobalt, magnesium, and manganese content of control and lactocin 27 treated cells of *L. helveticus* strain LS18 was determined by neutron activation analysis. The bacteriocin did not affect divalent ions;

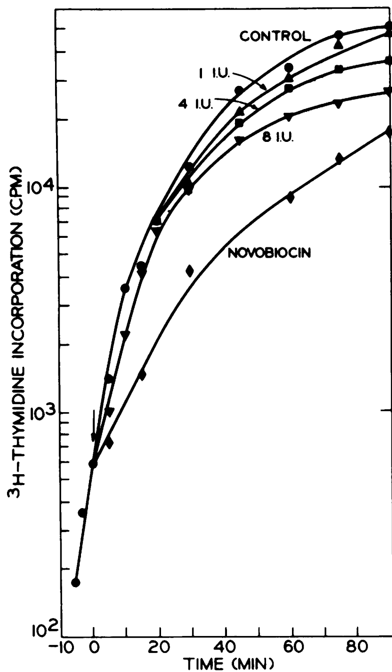


FIG. 4. *Effect of lactocin 27 on incorporation of [³H]thymidine. Buffer was used in case of control and novobiocin (80 μg/ml), a known inhibitor of DNA synthesis, was used for comparison. The unexpected incorporation of [³H]thymidine (0.5 μCi/ml) in the presence of novobiocin might be attributed to the acidic pH which could cause the novobiocin to become insoluble. Naladixic acid and mitomycin C were not inhibitory. [³H]thymidine (0.5 μCi/ml) was added at T-5 min and lactocin (or buffer or novobiocin) was added at $T_0$ (indicated by arrow).*

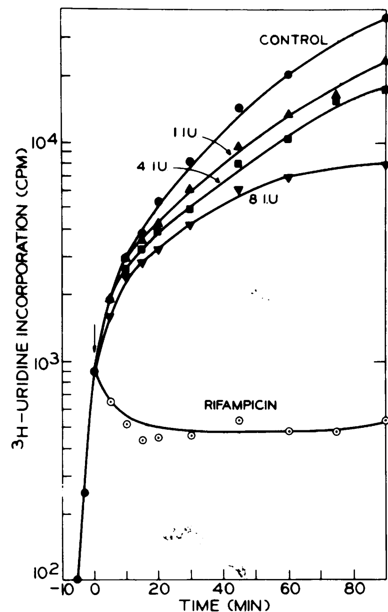


FIG. 5. *Effect of lactocin 27 on incorporation of [³H]uridine. Buffer was used in case of control and rifampin (20 μg/ml), a known inhibitor of RNA synthesis, was used for comparison. [³H]uridine (0.5 μCi/ml) was added at T-5 min and lactocin (or buffer or rifampin) was added at $T_0$ (indicated by arrow).*

however it caused leakage of potassium ions and an initial influx of sodium ions (Fig. 7).

**Adsorption of lactocin.** The adsorption of lactocin 27 to *L. helveticus* strain LS18 could be shown only if the final concentration of lactocin 27 was 2 I.U. or less. At this concentration (2 I.U.), however, lactocin 27 adsorbed to all the microorganisms tested and thus showed no specificity.

## DISCUSSION

Lactocin 27, when isolated from a broth culture of *L. helveticus* strain LP27, was obtained as a non-dialyzable, large-molecular-weight complex (20). However, when *L. helveticus* strain LP27 cells are grown over a thin dialysis membrane on agar, at least a portion of the inhibitor diffuses into the agar below (Fig. 1). It is possible that in broth culture the lactocin is first produced as a small-molecular-weight

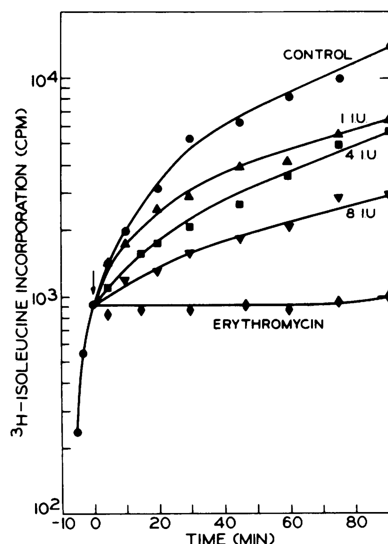


FIG. 6. *Effect of lactocin 27 on incorporation of [³H]isoleucine. Buffer was used in case of control and erythromycin (80 μg/ml), a known protein synthesis inhibitor, was used for comparison. [³H]isoleucine (1 μCi/ml) was added at T-5 min and lactocin (or buffer or erythromycin) was added at $T_0$ (indicated by arrow).*

TABLE 1. *Tests for the specific incorporation of [³H]thymidine into DNA*[a]

| Determinants | Control | | Acid hydrolyzed | | Alkali hydrolyzed | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Buffer only | 12,632 | 33,006 | 313 | 416 | 12,319 | 31,974 |
| Lactocin (1 I.U.) | 11,249 | 30,136 | 356 | 392 | 10,237 | 29,826 |
| Lactocin (4 I.U.) | 9,987 | 27,301 | 364 | 387 | 9,286 | 27,427 |
| Lactocin (8 I.U.) | 9,784 | 21,678 | 401 | 396 | 9,352 | 20,214 |
| Novobiocin (80 µg/ml) | 6,238 | 10,927 | 298 | 319 | 5,986 | 10,524 |

[a] Results shown in counts per minute.

TABLE 2. *Tests for the specific incorporation of [³H]uridine into RNA*[a]

| Determinants | Control | | Acid hydrolyzed | | Alkali hydrolyzed | |
|---|---|---|---|---|---|---|
| | 30 min | 60 min | 30 min | 60 min | 30 min | 60 min |
| Buffer only | 8,138 | 21,990 | 7,984 | 20,932 | 136 | 139 |
| Lactocin (1 I.U.) | 6,241 | 14,235 | 5,984 | 13,966 | 152 | 160 |
| Lactocin (4 I.U.) | 5,137 | 11,188 | 4,893 | 10,412 | 184 | 178 |
| Lactocin (8 I.U.) | 4,426 | 6,903 | 4,387 | 6,799 | 167 | 158 |
| Rifampin (20 µg/ml) | 461 | 487 | 427 | 418 | 92 | 94 |

[a] Results shown in counts per minute.

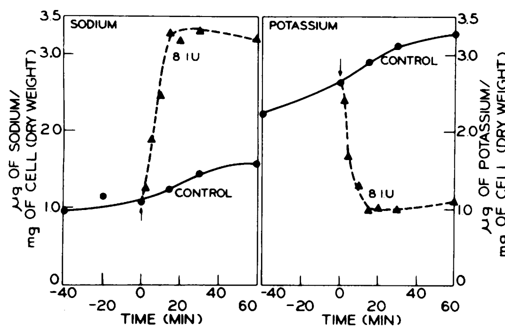


FIG. 7. *Effect of lactocin 27 on intracellular sodium and potassium of L. helveticus strain LS18. Lactocin (buffer in case of control) was added at* $T_0$ *(indicated by arrows).*

entity and then conjugates with other cell components, resulting in a large-molecular-weight complex. A similar situation has also been reported in the case of staphylococcin 462 (10) and colicins, which exist as small-molecular-weight, simple proteins and large-molecular-weight lipoprotein-carbohydrate complexes (17). Unlike colicins however, the lactocin production could not be induced by UV light or mitomycin C (20).

In the freshly subcultured *L. helveticus* strain LP27, the loss of lactocin production was negligible. On a prolonged storage for approximately 1 year, roughly 50% of the CFU lost their ability to produce lactocin. Jetten and Vogels (13) made a systematic time study of the spontaneous loss of staphylococcin production in three strains of *Staphylococcus aureus* and detected the spontaneous loss (>80%) of staphylococcin production in two strains within 1 year's time. Loss of lactocin production through storage of cultures has not been studied systematically. The treatment by potential curing agents and also the growth at 42 C did not cure lactocin production. These treatments however cured the staphylococcin production (5, 13). These findings suggest that the lactocin production is not controlled by an extrachromosomal plasmid. Because of our lack of knowledge of genetic markers and transduction phages in lactobacilli, this point could not be confirmed at present.

The gross effects of lactocin 27 on *L. helveticus* strain LS18 are not fully understood, but seem likely to represent stasis rather than killing. As shown in Fig. 2, sufficient lactocin (10 I.U. or more) inhibits CFU almost completely, presumably because sufficient lactocin is present with or on the cells, even after the usual dilution, to inhibit growth. With lesser amounts, 4 and 8 I.U. (Fig. 3), there is an abrupt drop in CFU followed by a partial recovery which remains unexplained. With 8 I.U. there is no increase in CFU during the second hour and only a slight increase in absorbance which may be due to extracellular products rather than an

increase in cells. Thus, it appears the cells are prevented from growth while in contact with the bacteriocin. Treated cells when diluted into fresh broth without lactocin 27 behave normally, with both CFU and absorbance increasing at the rate of untreated cells. These cells, if treated with lactocin 27, behave like the parent culture and thus do not represent unsusceptible cells.

The view that lactocin is primarily bacteriostatic is entirely consistent with the findings of the study on macromolecular synthesis. Protein synthesis was slowed almost immediately, apparently without interferring with DNA and RNA synthesis. The inhibition was independent of energy metabolism as ATP synthesis was not inhibited. Lactocin 27 did not damage the cell membrane in a generalized way, as UV-absorbing materials were not released. However, it caused a leakage of potassium ions and an influx of sodium ions. Thus, the biochemical effect(s) of lactocin 27 are somewhat similar to cloacin DF13 (7), except for the inhibition of ATP synthesis. It should be remembered, however, that in homofermentative lactobacilli, the ATP is generated solely by substrate level phosphorylation, as opposed to *Enterobacter cloaceae* where oxidative phosphorylation is the predominant source of ATP.

The adsorption of lactocin 27 could only be demonstrated at a maximum of 2 I.U. Certain other bacteriocins must also be used in low concentrations if adsorption is to be demonstrated (6, 11). At this concentration, however, lactocin 27 adsorbs non-specifically to various microorganisms irrespective of their susceptibility. The nonspecific adsorption, although uncommon for most bacteriocins, has also been shown in case of staphylococcin 414 (9) and staphylococcin 1580 (12). For lactocin 27, the non-specificity of adsorption might be attributed to the presence of lipid and carbohydrate. Therefore, the presence of specific receptors on the susceptible *L. helveticus* strain LS18 cells cannot be ruled out at present. The loss of potassium ions due to lactocin 27 treatment suggests that it acts on the cell membrane. It is possible that like colicin $E_3$ and cloacin DF13, the lactocin 27 may penetrate to the level of its biochemical target(s). The question of specific adsorption might be answered if it were possible to isolate lactocin 27 as unconjugated protein free of SDS.


## ACKNOWLEDGMENTS

We wish to express our appreciation to R. J. Cashwell, Nuclear Engineering Department, Univ. of Wisconsin, Madison, for the neutron activation analyses. We also thank Sue Keller and Donald Sauch for their excellent technical help, and Walter Leps for ATP estimations.

This research was supported in part by Public Health Service grant AI 08737 from the National Institute of Allergy and Infectious Diseases.


## LITERATURE CITED


1. Boon, T. 1971. Inactivation of ribosomes *in vitro* by Colicin E3. Proc. Nat. Acad. Sci. U.S.A. **68**:2421-2425.
2. Boon, T. 1972. Inactivation of ribosomes *in vitro* by colicin E3 and its mechanism of action. Proc. Nat. Acad. Sci. U.S.A. **69**:549-552.
3. Bowman, C. M., J. Sidikaro, and M. Nomura. 1971. Specific inactivation of ribosomes of Colicin E3 *in vitro* and mechanism of immunity in colicinogenic cells. Nature (London) **234**:133-137.
4. Cramer, W. A., S. K. Phillips, and T. W. Keenan. 1973. On the role of membrane phase in the transmission mechanism of Colicin E1. Biochemistry **12**:1177-1181.
5. Dajani, A. S., and Z. Taube. 1974. Plasmid mediated production of staphylococcin in bacteriophage Type 71 *Staphylococcus aureus*. Antimicrob. Agents Chemother. **5**:594-598.
6. Dajani, A. S., and L. W. Wannamaker. 1973. Kinetic studies on the interaction of bacteriophage type 71 staphylococcal bacteriocin with susceptible bacteria. J. Bacteriol. **114**:738-742.
7. DeGraff, F. K. 1973. Effects of cloacin DF13 on the functioning of the cytoplasmic membrane. Antonie van Leeuwenhoek J. Microbiol. Serol. **39**:109-119.
8. DeKlerk, H. C., and J. A. Smit. 1967. Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. **48**:309-316.
9. Gagliano, V. J., and R. D. Hinsdill. 1970. Characterization of a *Staphylococcus aureus* bacteriocin. J. Bacteriol. **104**:117-125.
10. Hale, E. M., and R. D. Hinsdill. 1973. Characterization of a bacteriocin from *Staphylococcus aureus* strain 462. Antimicrob. Agents Chemother. **4**:634-640.
11. Ivanovics, G. 1962. Bacteriocins and bacteriocin-like substances. Bacteriol. Rev. **26**:108-118.
12. Jetten, A. M., and G. D. Vogels. 1972. Mode of action of a *Staphylococcus epidermidis* bacteriocin. Antimicrob. Agents Chemother. **2**:456-463.
13. Jetten, A. M., and G. D. Vogels. 1973. Characterization and extrachromosomal control of bacteriocin production in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **4**:49-57.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
15. Nomura, M. 1967. Colicins and related bacteriocins. Annu. Rev. Microbiol. **21**:257-284.
16. Plate, C. A. 1973. Effects of temperature and of fatty acid substitutions on Colicin K action. Antimicrob. Agents Chemother. **4**:16-24.
17. Reeves, P. 1972. The bacteriocins, p. 1-80. *In* A. Kleinzeller, G. F. Springer, and H. G. Wittmann (ed.), Molecular biology, biochemistry and biophysics, vol. 11. Springer-Verlag, New York.
18. Ringrose, P. S. 1972. Interaction between colicin E2 and DNA *in vitro*. FEBS Lett. **23**:241-243.
19. Sidikaro, J., and M. Nomura. 1973. Colicin E3 induced *in vitro* inactivation of ribosomes from colicin insensitive bacterial species. FEBS Lett. **29**:15-19.
20. Upreti, G. C., and R. D. Hinsdill. 1973. Isolation and characterization of a bacteriocin from a homofermentative *Lactobacillus*. Antimicrob. Agents Chemother. **4**:487-494.