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NOTES

Bacteriocin Production by Group A Streptococcal L-Forms

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L-forms induced from a bacteriocin-producing strain of group A streptococcus retained both the ability to produce the streptococcin and producer strain immunity to the homologous bacteriocin. L-forms of a spontaneously cured (bacteriocin negative) derivative of this same strain failed to produce streptococcin but were sensitive to its action.

Streptococcin A-FF22 (SA) is a bacteriocin produced by group A streptococcus strain FF22 (M type 52). Characterization and partial purification of this bacteriocin have been described previously (7, 8). Recent results (Tagg, unpublished observations) indicate that SA may exist in two forms: firmly bound to the cell envelope or as an extracellular product. Studies of bacteriocin production by L-forms have been few in number, and none of these have related to group A streptococcal L-forms (1). Among gram-negative bacteria, the effect of cell wall loss on susceptibility to colicins is a controversial subject, with some studies indicating that the cell wall is necessary for attachment of these bacteriocins (4) and others indicating that the cell membrane carries the specific receptors (5).

It was of interest to determine whether L-forms of group A streptococcus strain FF22 are capable of bacteriocin production and also whether loss of cell wall results in increased sensitivity to SA.

Group A streptococcus L-forms of strain FF22 (SA^+) and of strain SPON1 (SA^-), a non-SA-producing spontaneously cured derivative, were induced with group C streptococcal phage-associated lysin (muralysin), using the method described by Maxted (3). The induction medium was composed of brain heart infusion (Difco) supplemented with 10% horse serum, 25% sucrose, and 1.2% agar (Difco). L-form induction and the first few passages were in the presence of penicillin (10 IU/ml), but subsequent propagation was on penicillin-free medium to eliminate this possible source of non-bacteriocin inhibition.

After 3 to 5 days of incubation at 37°C, morphologically typical L-forms developed and were maintained by the technique of agar block transfers. After 10 to 15 passages, stable L-forms were established as evidenced by their failure to revert to bacterial forms on penicillin-free L-form medium and on blood agar medium. Occasional spontaneous reversion was observed during the first few passages on penicillin-free medium or blood agar. The revertants were characterized as identical to the parent form by morphology on blood agar, staining, and serological grouping and typing. Tests (7, 8) of the bacteriocin produced in tryptic soy broth cultures by the revertant and by the parent strain indicated a similar titers and activity spectra.

Four agar blocks containing stable L-forms from two independent inductions of the SA^+ strain and of its SA^- derivative were placed on penicillin-free L-form agar medium and incubated for 48 h. The blocks were removed, and L-form growth in the absence of bacterial forms was confirmed. The plate was exposed to chloroform vapors to kill surface colonies (8) and then overlayered with an indicator layer of soft agar containing *Staphylococcus aureus* strain CIT (6). After overnight incubation at 37°C, a wide zone of inhibition was observed around the L-form colonies derived from the SA^+ strain (Fig. 1). The SA^- L-form colonies were not inhibitory. To eliminate inhibitory effects due to residual penicillin, which might have been present in the agar block, penicillinase (Bacto Penase, Difco) was included in the L-form me-

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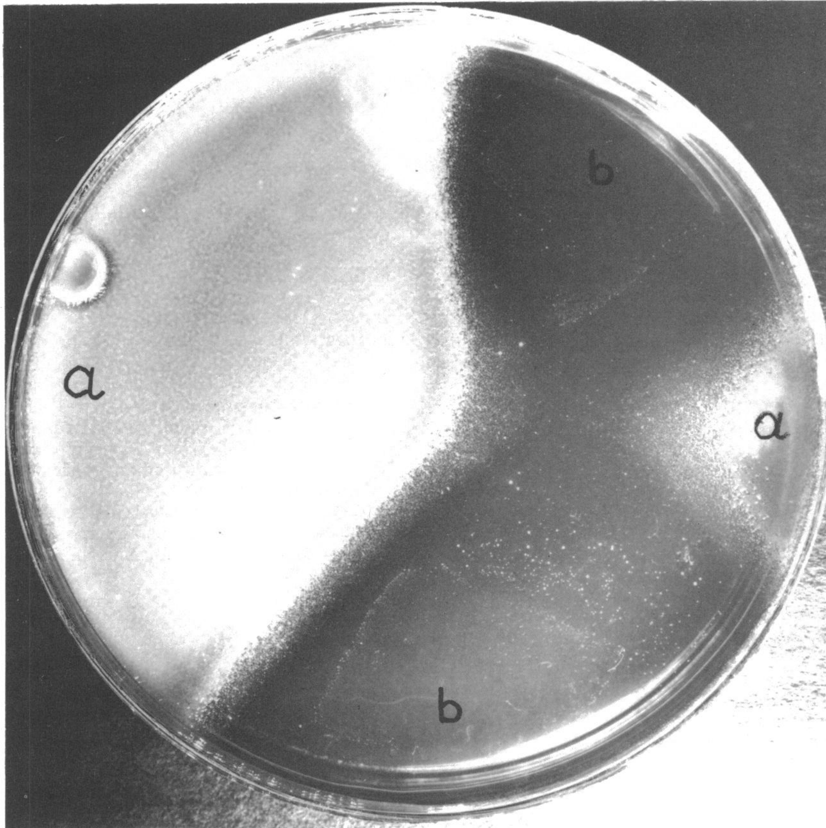

FIG. 1. *Inhibition of Staphylococcus aureus CIT by bacteriocin-producing streptococcal group A L-forms. (a) S. aureus CIT; (b) streptococcal L-forms (SA⁺).*

dium. No reduction in inhibitory effect was observed with the inclusion of penicillinase.

The bacteriocin sensitivity of SA^+ and SA^- L-forms was tested by incorporating partially purified streptococcin (8) into L-form medium in the concentrations indicated in the table. L-forms were allowed to grow on bacteriocin-containing agar medium for 48 h, and then the parent bacterial strains were spread onto adjacent areas of the same plate. No loss of bacteriocin activity occurred under the test conditions. Group A streptococcus strain FF22 (SA^+) and its L-form derivative appeared resistant to the bacteriocin (Table 1). By comparison, both the cured strain and its L-form were considerably more sensitive to the streptococcin. Application of studies in liquid medium should provide more precise information.

From these data we conclude that L-forms induced from an SA^+ group A streptococcus retain the ability to produce bacteriocin. Other reported studies (2) have shown that group A streptococcal L-forms still produce M protein and various other extracellular products that are elaborated by the parent streptococcus. It is apparent that retention of an intact cell wall is not essential for SA production. Moreover, producer strain immunity to the homologous bacteriocin seems to be preserved in the L-form phase of group A streptococcus strain FF22.

TABLE 1. *Effect of streptococcin A-FF22 (SA) on growth of SA^+ and SA^- streptococci and L-forms*

Organism	Growth at SA concn (U/ml of medium)[a] of:		
	64	32	16
Strain FF22 (SA^+)	+++[b]	+++	+++
L-form derivative (SA^+)	+++	+++	+++
Strain SPON1 (SA^-)	+	±	−
L-form derivative (SA^-)	++	−	−

[a] Streptococcin units are as defined previously (8).
[b] Symbols: ±, 1 to 5 colonies; +, 6 to 10 colonies; ++, 11 to 50 colonies; +++, over 50 colonies.

The SA^- derivative L-forms seem to retain the sensitivity of the parent bacterial form to the bacteriocin, the implication being that the cell wall may not be necessary to effect the bactericidal action of SA.

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LITERATURE CITED

1. **Harwick, H. J., J. Z. Montgomerie, G. M. Kalamanson, E. G. Hubert, C. S. Potter, and L. B. Guze.** 1971. Differential action of a streptococcal bacteriocin on mycoplasmas and microbial L-forms. Infect. Immun. 4:194-198.
2. **Maxted, W. R.** 1964. Streptococcal bacteriophages, p. 25-44. *In* J. W. Uhr (ed.), The streptococcus, rheumatic fever and glomerulonephritis. The Williams & Wilkins Co., Baltimore.
3. **Maxted, W. R.** 1972. Specific procedures and requirements for the isolation, growth and maintenance of the L-phase of some microbial groups, p. 423-449. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 7A. Academic Press Inc., New York.
4. **Sabet, S. F., and C. A. Schnaitman.** 1971. Localization and solubilization of colicin receptors. J. Bacteriol. 108:422-430.
5. **Smarda, J., and U. Taubeneck.** 1968. Situation of colicin receptors in surface layers of bacterial cells. J. Gen. Microbiol. 52:161-172.
6. **Tagg, J. R., A. S. Dajani, and L. W. Wannamaker.** 1975. Bacteriocin of a group B streptococcus: partial purification and characterization. Antimicrob. Agents Chemother. 7:764-772.
7. **Tagg, J. R., A. S. Dajani, L. W. Wannamaker, and E. D. Gray.** 1973. Group A streptococcal bacteriocin. Production, purification and mode of action. J. Exp. Med. 138:1168-1183.
8. **Tagg, J. R., R. S. D. Read, and A. R. McGiven.** 1973. Bacteriocin of group A streptococcus: partial purification and properties. Antimicrob. Agents Chemother. 4:214-221.