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Cerulenin-Inhibited Cells of *Staphylococcus aureus* Resume Growth When Supplemented with Either a Saturated or an Unsaturated Fatty Acid

ROBERT A. ALTENBERN

Pathology Division, United States Army Medical Research Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

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In the presence of an inhibitory concentration of cerulenin, cells of *Staphylococcus aureus* can resume growth when supplemented with either a saturated or an unsaturated fatty acid. A requirement for both types of acids for growth could not be demonstrated.

The antibiotic cerulenin has been shown to inhibit, reversibly, fatty acid biosynthesis by interfering with the action of β-ketoacyl-acyl carrier protein synthetase (4). Several studies (5, 8) have demonstrated that both a saturated fatty acid (SFA) and an unsaturated fatty acid (UFA) or branched-chain saturated acid are required to reverse the inhibition by cerulenin of either *Escherichia coli* or *Bacillus subtilis*. Although the minimal inhibitory concentration of cerulenin for *Staphylococcus aureus* (6) and the failure of cerulenin to block induction of tetracycline resistance in *S. aureus* (2) have been reported, no data on restoration of growth of *S. aureus* cells inhibited by cerulenin have appeared. In this article, it is shown that although an SFA and a UFA together do promote growth of cerulenin-inhibited cells of *S. aureus*, single supplementation with certain SFAs or UFAs permits resumption of growth in the presence of cerulenin.

S. aureus ATCC 14458 was grown in NAK medium (1) at 37°C on a shaker for 24 h. Tubes (18 by 150 mm) containing 5 ml of NAK medium plus graded amounts of cerulenin (Makor Chemicals, Ltd., Jerusalem, Israel) and supplemented with various concentrations of SFAs and/or UFAs were inoculated with 0.05 ml of the 24-h culture. The tubes were then incubated at an angle on a shaker at 37°C. At 30-min intervals, the absorbance at 600 nm was measured on a Coleman Jr. spectrophotometer to determine mass doubling time(s). Subsequently, the absorbance of a 1:5 dilution in water of 21-h cultures measured the final growth density. There was no attempt made to determine colony-forming units per milliliter during exponential growth or at the final density attained. It is possible that under the varied fatty acid supplementation reported in this note, cell size and cell viability may affect the number of colony-forming units per unit of optical density. Cerulenin and fatty acids were added as ethanolic solutions. All fatty acids were purchased from Sigma Chemical Co., St. Louis, Mo.

Preliminary experiments (Fig. 1) showed that the minimal inhibitory concentration of cerulenin for this strain of *S. aureus* was about 100 μg/ml, in good agreement with 100 μg/ml reported by Omura et al. (6) for another strain and 125 μg/ml used by Chopra (2).

The effect of graded amounts of oleic acid in the presence of several constant levels of pal-

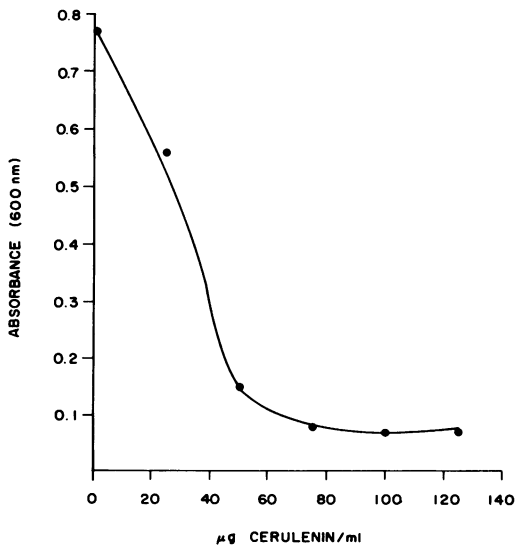

FIG. 1. *Final growth density of a 1:5 dilution of S. aureus in the presence of various concentrations of cerulenin.*

mitic acid on final growth density of *S. aureus* strain 14458, in NAK medium containing 100 μg of cerulenin per ml, is presented in Fig. 2. The concentration levels of palmitic acid were 8, 24, and 40 μg/ml. Oleic acid was added so that the ratios of SFAs to UFAs (micrograms per milliliter) were 25, 8, 2, and 1 at each level of the palmitic. There was no evidence in this and other experiments that there was an optimal ratio for growth. It is clear that with a constant level of palmitate, oleic acid in increasing amounts promoted greater growth. Similar experiments with palmitic plus elaidic acids or stearic plus oleic acids yielded nearly identical results. Of interest was the observation that palmitic acid alone supported considerable growth, and that the final growth density was a function of palmitic acid concentration.

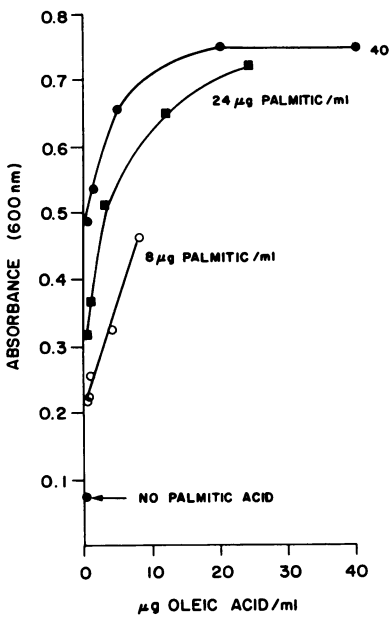

FIG. 2. *Growth of S. aureus in cerulenin medium containing 8, 24, or 40 μg of palmitic acid per ml and graded concentrations of oleic acid.*

Consequently, the ability of various SFAs or UFAs to support growth of *S. aureus* in NAK medium containing 100 μg of cerulenin per ml was determined (Fig. 3). Only palmitic and stearic acids of the SFAs, with the possible exception of arachidic acid, allowed appreciable growth in the presence of cerulenin. Petroselinic, elaidic, and oleic acids were the only UFAs stimulating growth. Such UFAs as arachidonic, palmitoleic, and docosahexaenoic acid were growth inhibitory in the lowest concentration tested (1.6 μg/ml).

Determination of mass doubling times from regular measurements of absorbance during exponential growth showed that most rapid growth was supported by palmitic acid, followed by stearic, arachidic, and the UFAs (Table 1). It appears that only those acids with a 16-, 18-, or 20-carbon chain were effective.

In almost all respects, the response of *S. aureus* to cerulenin inhibition closely matched results obtained with *E. coli* (5) and *B. subtilis* (8). For *S. aureus*, inoculation of media containing 100 μg of cerulenin per ml resulted in a

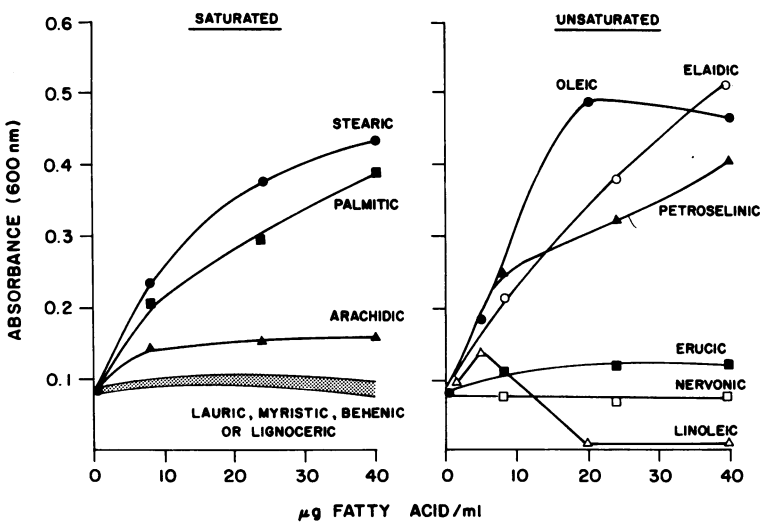

FIG. 3. *Support of growth of S. aureus in cerulenin medium by single supplementation with SFAs or UFAs.*

TABLE 1. *Mass doubling time for S. aureus growing in medium containing cerulenin and certain fatty acids*[a]

Fatty acid	Mass doubling time (min) in fatty acid (μg/ml):					
	1.6	5.0	8.0	20	24	40
Palmitic			82		70	76
Stearic			128		103	85
Arachidic			115		115	115
Elaidic			133		135	128
Petroselinic			133		154	154
Oleic	153	155		130		150

[a] Mass doubling time in unsupplemented medium was 42 min.

slow increase in absorbance up to about 3 h of incubation, at which time all growth ceased. The presence of effective fatty acids allowed growth at a maximum rate of 50 to 60% of the rate in cerulenin- and fatty acid-free medium.

It is concluded that *S. aureus* can grow in the minimal inhibitory concentration of cerulenin when supplemented with either palmitic or stearic acid and can dispense with the expected requirement for a UFA. The C_{15}, C_{17}, and C_{19} iso or ante-iso fatty acids comprise at least 85% of the total fatty acids of the membrane phospholipids of *S. aureus* grown under commonly used conditions (7) and seem to confer the desired degree of fluidity to the membrane lipids, since the amount of straight-chain SFAs or UFAs is minimal. It is estimated that *E. coli* requires for growth a minimum of 10% of the total fatty acid supplement as a SFA or UFA (3). Such a requirement cannot be demonstrated for *S. aureus* with the spectrum of acids used in this study. It is possible that *S. aureus* can carry out oxidative desaturation of palmitic or stearic acids to satisfy a hidden requirement for UFA or, conversely, that UFAs can be reduced to furnish a saturated acid. However, identical results were obtained when incubation was carried out statically and without aeration, although final growth densities were considerably lower than those for aerated (shaken) cultures. In any event, *S. aureus* does not display a stringent requirement for an exogenous supply of both an SFA and a UFA for growth in the presence of cerulenin.

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