Fatty Acids and Monoacylglycerols Inhibit Growth of *Staphylococcus aureus*

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ABSTRACT: *Staphylococcus aureus* causes a variety of human infections including toxic shock syndrome, osteomyelitis, and mastitis. Mastitis is a common disease in the dairy cow, and *S. aureus* has been found to be a major infectious organism causing mastitis. The objectives of this research were to determine which FA and esterified forms of FA were inhibitory to growth of *S. aureus* bacteria. FA as well as their mono-, di-, and triacylglycerol forms were tested for their ability to inhibit a human toxic shock syndrome clinical isolate (MN8) and two *S. aureus* clinical bovine mastitis isolates (305 and Novel). The seven most potent inhibitors across all strains tested by minimum inhibitory concentration analysis included lauric acid, glycerol monolaurate, capric acid, myristic acid, linoleic acid, *cis*-9, *trans*-11 conjugated linoleic acid, and *trans*-10, *cis*-12 conjugated linoleic acid. Some of these lipids were chosen for 48-h growth curve analysis with a bovine mastitis *S. aureus* isolate (Novel) at doses of 0, 20, 50, and 100 μg/mL except myristic acid, which was tested at 0, 50, 100, and 200 μg/mL. The saturated FA (lauric, capric, myristic) and glycerol monolaurate behaved similarly and reduced overall growth. In contrast, the polyunsaturated FA (linoleic and *cis*-9, *trans*-11 conjugated linoleic acid) delayed the time to initiation of exponential growth in a dose-dependent fashion. The results suggest that lipids may be important in the control of *S. aureus* during an infection.

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Staphylococcus aureus is a broad host range pathogen and a leading cause of infections in both humans and domesticated animals worldwide. Infections caused by *S. aureus* are extremely common and often life threatening, creating the potential for *S. aureus* to increase morbidity and mortality. *S. aureus* causes nearly any type of human infection including toxic shock syndrome (TSS), osteomyelitis, and mastitis (1,2). *S. aureus* also causes mastitis in dairy cattle, which is the most economically important disease to the dairy industry in the United States (3). Mastitis is also a common disease during human lactation, affecting 20% to 33% of women in developed and developing countries (4,5). *S. aureus* has been found to be the main infectious organism in cases of human mastitis (6,7).

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Abbreviations: CLA, conjugated linoleic acid; GML, glycerol monolaurate; MIC, minimum inhibitory concentration; OD_{600}, optical density at 600 nm; TSB, tryptic soy broth; TSS, toxic shock syndrome.

Early reports (8,9) have characterized the ability of lipids to inhibit bacteria. Various FA and monoacylglycerols in trace amounts have an inhibitory effect on the growth of microorganisms (10). Both bacteriostatic (8,9,11) and bactericidal effects (8,12,13) have been observed. Lauric acid and glycerol monolaurate (GML) have been extensively characterized in their ability to inhibit the growth and toxin production of *S. aureus* (14,15). Other FA in milk have also been shown to inhibit bacterial growth including capric acid, myristic acid, linoleic acid, and linolenic acid. This inhibition of growth by FA has been demonstrated in several species of bacteria including *S. aureus*, *Streptococcus pneumoniae*, and *Streptococcus* group A (10,16). One of the hallmark signs of an infection caused by *S. aureus* bacteria is a release of FFA in the infected area as observed in intraperitoneal abscesses, skin infections, and mastitis across a variety of animal models (17–20). Increased concentrations of FFA in milk are common during mastitis in cows (21). Increases of 50% to 100% have been detected in FFA content of milk from cows with mastitis (22–26). However, despite the impact of mastitis on the health of the cow or woman, little is known about the effect of these FA on bacteria during an infection. To begin to investigate this, we hypothesized that certain FA and monoacylglycerols commonly found in milk would inhibit growth of *S. aureus*.

EXPERIMENTAL PROCEDURES

Bacterial strains and growth conditions. The bacterial strains used in this study represent two different human diseases caused by *S. aureus* and include *S. aureus* MN8, a well-characterized human TSS isolate (27), and two clinical bovine mastitis isolates, *S. aureus* Novel (28) and *S. aureus* 305 (29). Cultures of each strain were prepared by inoculating a single colony into 3 mL of tryptic soy broth (TSB) (VWR, Gibbstown, NJ, USA) before incubation at 37°C overnight with shaking at 250 rpm. All minimum inhibitory concentrations (MIC) were done in duplicate and cultured in 3 mL of TSB containing a serial dilution of each particular FA. Lipids (Sigma, St. Louis, MO, USA) were solubilized in ethanol before adding to TSB to get a final concentration of 0, 12.5, 25, 50, 100, and 200 μg/mL. A control tube containing TSB and an equivalent amount of ethanol was included. Culture tubes were inoculated 1:100 with the overnight culture of the *S. aureus* strain tested and incubated 24 h with continuous shaking

at 250 rpm to determine if growth occurred. The minimum concentration necessary to prevent turbid growth was considered the MIC and evaluated visually. Growth curves were performed in triplicate and used tryptic soy broth (25 mL) inoculated 1:100 with overnight culture of *S. aureus* Novel, adjusted to an optical density at 600 nm (OD_{600}) of 0.1 units. The cultures were allowed to grow for 2 h with continuous shaking at 250 rpm to reach the growth phase. Lipids including lauric acid, GML, capric acid, myristic acid, linoleic acid, and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) were then added at 0, 20, 50, and 100 μg/mL concentrations (except myristic acid, which was tested at 0, 50, 100, and 200 μg/mL) and the incubation was continued at 37°C with continuous shaking at 250 rpm. Samples were taken frequently, and the OD_{600} was read after appropriate dilution (whenever the absorbance exceeded a value of 1.0, dilutions were made until the value was within the linear range, and the absorbance value was multiplied by the dilution factor to obtain the OD_{600} value) using a Perkin-Elmer MBA2000 spectrophotometer (Boston, MA, USA). We found 1 OD_{600} unit is equivalent to 2.25 ± 0.29 mg/mL dry weight of *S. aureus* Novel.

Statistics. Minimum inhibitory concentrations were conducted twice and reported as the mean of duplicate runs. Statistical analysis of growth curves used the typical parabolic pattern of bacterial population growth. Interestingly, in some of our experimental cultures, we observed a lag phase representing delay in onset of increased growth followed by a parabolic curve. The ideal description of bacterial growth in our system, therefore, should include both the linear lag phase and the parabolic growth phase in a jointed regression, allowing estimation of the joint points and the maximum growth achieved. The fitted model was of the form:

$$Y = \beta_o + \beta_1 t + \beta_2 t^2 \text{ for } t > t_o$$
$$Y = c \text{ for } t \leq t_o$$

where Y is the OD_{600}, t is time in h, t_o is the time joint point is reached (lag before exponential growth), c is a constant representing the value of OD_{600} during the lag phase, and β_o, β_1, and β_2 are regression coefficients associated with the intercept, slope, and inflection (maximum) point, respectively. A generalized nonlinear estimated algorithm was used for parameter estimation. Separate models were estimated for each of the FA tested. The adequacy of fit was determined by the significance of the parameter estimates, and their corresponding signs and magnitudes, examination of the model residuals, and the structure of correlation among estimated parameters. A full model dummy variable regression procedure was used to conduct preplanned contrasts to compare coincidence (equality) of estimated lines, joint points, and maximum values at specific doses. Contrasts of estimated lines included all parameter estimates. Significance was declared at $P < 0.05$. Statistical computations were performed using the PROC NLMIXED procedure of SAS (30).

RESULTS

Minimum inhibitory concentrations. The MIC of the major FA present in milk along with several of their monoacylglycerols, diacylglyerols, and triacylglycerols forms were determined for two *S. aureus* bovine mastitis isolates (strain 305 and Novel), as well as the TSS isolate, MN8 (Table 1). The lipids that were the most inhibitory were similar for all three strains (except oleic acid was not inhibitory to strain MN8) and included lauric acid, glycerol monolaurate (GML), linoleic acid, *cis*-9, *trans*-11 conjugated linoleic acid (CLA), *trans*-10, *cis*-12 CLA, capric acid, and myristic acid. As shown in Table 1, the majority of FFA and their triglycerides from C6:0 to C18:0 did not inhibit growth at the maximum concentration tested (200 μg/mL) except stearic acid, which was inhibitory to the Novel strain at 200 μg/mL.

Growth curves. Several of the FA shown to have inhibitory activity were further examined in batch cultures of the *S. aureus* Novel strain including capric acid, myristic acid, lauric acid, GML, linoleic acid, and *cis*-9, *trans*-11 CLA. Growth curves were performed using concentrations of 0, 20, 50, and 100 μg/mL, with the exception of myristic acid, which was tested at 0, 50, 100, and 200 μg/mL. Parameter estimates were significant, suggesting the models were reasonable and all parameters were required. The correlation among parameters was also examined and most showed < 0.95 correlation, indicating a lack of redundancy among parameters. For each dose of FA tested, the model followed data trends (data for *cis*-9, *trans*-11 CLA presented in Fig. 1). The expected lag phase followed by a parabolic curve was observed in all doses. Residuals were examined and found to be uniform and random with no discernible pattern (data not shown). The same procedure was used for all doses of the six FA tested, with very good agreement between the predicted model and the observed data (data not shown), similar to that presented for *cis*-9, *trans*-11 CLA.

Preplanned contrasts were conducted to compare growth curves, and we found that in the case of lauric acid, capric acid, linoleic acid, and *cis*-9, *trans*-11 CLA, growth curves at each dose differed from each other. In the case of GML, 0 μg/mL was not different than 20 μg/mL; however, the growth curves for 50 and 100 μg/mL differed from 0 and 20 μg/mL. In the case of myristic acid, the 200 μg/mL myristic acid growth curve differed from the growth curves for all other doses but the other growth curves were similar. To further describe how growth curves were different, contrasts of the joint points (measure of lag in the initiation of growth) and the maximum amount of growth achieved were conducted to compare these parameters at all doses.

When free lauric acid (50 and 100 μg/mL), a well-characterized inhibitory FA, was added to the growth medium (Fig. 2, top panel), the optical density of the culture increased initially but reached a maximum well below that seen in the untreated or 20 μg/mL lauric acid-supplemented cultures. Maximum growth of bacteria was 24.7 OD units at 0 μg/mL lauric acid and was re-

TABLE 1
Minimum Inhibitory Concentration (MIC) of Lipids on Strains of *Staphylococcus aureus*[a]

Lipid[b]	MN8	305	Novel
		MIC (μg/mL)	
C6:0 Caproic acid	NI	NI	NI
C8:0 Caprylic acid	NI	NI	NI
C10:0 Capric acid	100	100	100
C12:0 Lauric acid	50	50	50
Glycerol monolaurate	50	25	50
Glycerol dilaurate	100	NI	200
Glycerol trilaurate	NI	NI	NI
C14:0 Myristic acid	100	150	200
Glycerol monomyristate	200	200	200
Glycerol trimyristate	NI	NI	NI
C16:0 Palmitic acid	NI	NI	100
Glycerol monopalmitate	NI	NI	150
Glycerol dipalmitate	NI	NI	NI
Glycerol tripalmitate	NI	NI	NI
C17:0 Margaric acid	NI	NI	NI
C18:0 Stearic acid	NI	NI	200
C18:1 *cis*-9 Oleic acid	NI	100	100
C18:1 *trans*-9 Elaidic acid	NI	NI	NI
C18:1 *trans*-11 Vaccenic acid	NI	NI	NI
C18:2 *cis*-9, *cis*-12 Linoleic acid	100	50	50
Glycerol monolinoleate	200	100	200
Glycerol trilinoleate	NI	NI	NI
C18:2 *cis*-9, *trans*-11 CLA[c]	50	25	25
C18:2 *trans*-10, *cis*-12 CLA	100	50	75

[a]Numbers reported represent the mean of duplicates. NI = no inhibition observed at any dose tested.
[b]All FA tested were in FFA form. FA were esterified at the *sn*-1 position for all monoacylglycerols and at the *sn*-1 and *sn*-3 position for diacylglycerols.
[c]CLA = conjugated linoleic acid.

duced to 8.8 OD units ($P < 0.01$) at 100 μg/mL lauric acid (Table 2). Contrasts indicated that maximum growth was different at all doses of lauric acid (Table 3). These reduced values remained constant throughout the culture duration. The 20 μg/mL lauric acid had a higher maximum growth than 0 μg/mL lauric acid.

Glycerol monolaurate supplementation (Fig. 2, bottom panel, and Table 2) resulted in both a lag in the initiation of growth and a reduction in maximum growth achieved at 100 μg/mL compared to 0 μg/mL. The maximum growth yield achieved at 20 μg/mL GML was not different than maximum growth yield at 0 μg/mL GML ($P > 0.05$, Table 3). Maximum bacterial growth was different at all other doses of GML. There was a lag in the initiation of growth (joint point) at 50 and 100 μg/mL GML compared to 0 μg/mL GML.

For capric acid (Fig. 3, top panel, and Table 4), we observed a similar growth pattern to lauric acid. At 100 μg/mL capric acid there was a lower maximal growth than 0, 20, or 50 μg/mL capric acid ($P < 0.05$, Table 3). There was no difference in maximum growth at 0, 20, or 50 μg/mL capric acid. Although there was a lag in the initiation of growth at 20 vs. 50 μg/mL capric acid ($P < 0.01$, Table 3) and 0, 20, or 50 vs. 100 μg/mL capric acid ($P < 0.05$), there was very little practical difference between the joint point estimations of 0.51, 0.52, 0.56, and −0.26 OD units for 0, 20, 50, and 100 μg/mL capric acid, respectively. Myristic acid was also tested, but at slightly higher concentrations including 0, 50, 100, and 200 μg/mL based on the higher MIC value. The results of myristic acid supplementation were similar to capric acid (Fig. 3, bottom panel, and Table 4). Maximum growth was not different at any dose. However, the time that maximum growth was achieved was different at 200 μg/mL myristic acid than all other doses. The time when growth was initiated (joint point) did not differ between 0 and 100 μg/mL myristic acid.

When *cis*-9, *trans*-11 CLA was added, there was a dose-dependent lag in the initiation of bacterial growth (Fig. 4, bottom panel). Results indicated that the lag was 3.3 h with 20 μg/mL CLA and extended to 15.5 h with 100 μg/mL CLA (Table 5). Contrasts revealed that each of the joint points were different from each other (Table 3). To determine if the effect was similar to other long-chain polyunsaturated FA tested, cultures were grown with linoleic acid supplemented at the same concentrations. A similar dose-dependent lag in the initiation of growth occurred with linoleic acid (Fig. 4, top panel, and Table 5) as was observed for *cis*-9, *trans*-11 CLA. Results indicated that the lag in growth initiation at 50 μg/mL linoleic acid was different than the joint point at 0 μg/mL linoleic acid. The lag was extended with 100 μg/mL linoleic acid to 15.2 h, which was also different from 50 μg/mL linoleic acid where growth was initiated at 8.4 h. Maximum growth was not affected by either CLA or linoleic acid.

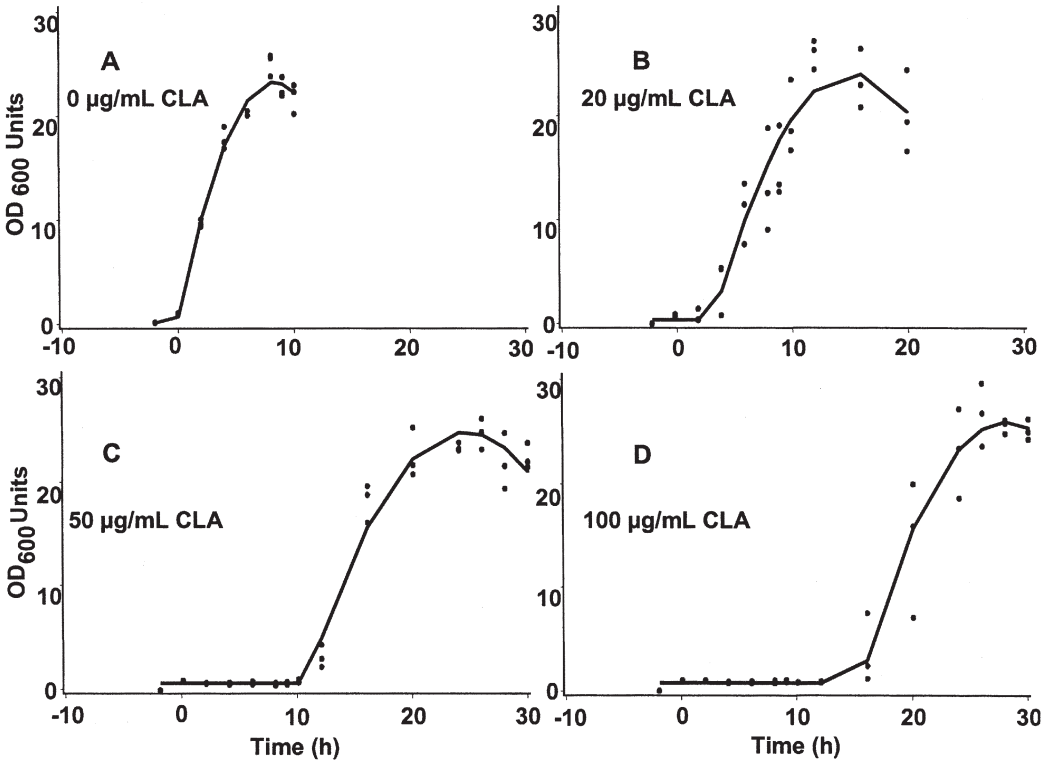

FIG. 1. Jointed nonlinear regression model fit to growth of *Staphylococcus aureus* Novel with 0 (A), 20 (B), 50 (C), or 100 µg/mL (D) of *cis*-9, *trans*-11 conjugated linoleic acid (CLA). Points are values for three replicates for each time of observation.

DISCUSSION

Fatty acids as antimicrobial agents. It has long been known that lipids have an inhibitory effect on bacteria (8,9). Trace amounts of FA have been shown to influence the growth of microorganisms in a very specific manner; certain FA such as lauric acid are more inhibitory than others (10). Kabara and coworkers (11) examined several specific straight-chain saturated FA and found lauric acid to be one of the most potent bacteriostatic FA when tested on gram-positive organisms in agreement with the present study results. They also investigated the effect of esterification and found that GML was the only monoacylglycerol more active than the FFA form, similar to our results (Table 1) where GML had the same or slightly lower MIC than lauric acid depending on the strain of *S. aureus* tested (11). The MIC of lauric acid (11) for *S. aureus* was higher (498 µg/mL) than what we observed (50 µg/mL). This may be due to differences in the sensitivity of *S. aureus* strains to certain lipids, as was found in the present study with 305 more sensitive than MN8 or Novel (Table 1). Kabara and coworkers (11) did not report what strain of *S. aureus* was used to determine their MIC (11). Our results indicate that certain FA and monoacylglycerols are more potent than others in their ability to inhibit bacterial growth similar to the previously described literature (8,9,12,13). In addition, we have demonstrated that *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA can inhibit growth of *S. aureus* (Table 1). We are not aware of previous reports on the effect of CLA on the growth of *S. aureus.*

We observed a very specific growth curve response when different FA were tested. The saturated FA, capric acid, myristic acid, and more dramatically, lauric acid, inhibited growth of bacteria through a decrease in the maximal amount of growth achieved. However, the polyunsaturated FA, linoleic, and *cis*-9, *trans*-11 CLA achieved inhibition through a dose-dependent lag in the initiation of growth. Once started, growth proceeded in a similar pattern to control. Previous reports with other species of bacteria including *Lactobacillus helveticus*, *Clostridium sporogenes,* and *Bacillus subtilis* observed similar growth inhibition with linoleic acid (10,31).

Our research suggests that some saturated and polyunsaturated FA inhibit the growth of *S. aureus* bacteria but by different mechanisms. It was also noted that in studies (10) with *Clostridium botulinum* that there may be different mechanisms of inhibition by saturated and unsaturated FA. Kodicek and Worden (32) characterized the action of unsaturated FA (i.e., linoleic acid) as bacteriostatic and reversible by surface-active agents like sterols and lecithin in *Lactobacillus* (32). Nieman (10) suggested that the inhibitory properties of FA appear more pronounced with increased chain length and degree of unsaturation (10). Knapp and Melly (12) found bactericidal effects on *S. aureus* mediated by peroxidation of arachidonic acid. Chamberlain and coworkers (33) demonstrated that addition of oleic

TABLE 2
Parameter Estimates for Growth of *Staphylococcus aureus* Novel Supplemented with Lauric Acid and Glycerol Monolaurate

		Lauric acid			Glycerol monolaurate		
Dose (μg/mL)	Parameter	Parameter estimate	Standard error	*P* value[a]	Parameter estimate	Standard error	*P* value
0	*c*	1.16	0.48	0.02	0.21	0.72	NS
	β_0	−4.54	1.35	<0.01	1.42	0.58	0.02
	β_1	8.16	0.61	<0.01	4.19	0.16	<0.01
	β_2	−0.59	0.06	<0.01	−0.21	0.01	<0.01
	Joint point[b]	0.74	0.18	0.01	−0.28	0.24	NS
	Time to max[c]	6.89	0.22	<0.01	9.96	0.15	<0.01
	Value at max[d]	24.72	0.59	<0.01	22.52	0.79	<0.01
20	*c*	1.18	0.90	NS	−0.01	0.86	NS
	β_0	−0.81	1.60	NS	1.58	0.69	0.03
	β_1	6.53	0.53	<0.01	4.21	0.19	<0.01
	β_2	−0.38	0.04	<0.01	−0.21	0.01	<0.01
	Joint point	0.31	0.27	NS	−0.37	0.26	NS
	Time to max	8.54	0.20	<0.01	10.26	0.21	<0.01
	Value at max	28.28	1.04	<0.01	23.18	0.95	<0.01
50	*c*	1.00	0.00	<0.01	0.44	0.33	NS
	β_0	1.80	0.64	<0.01	−9.90	1.40	<0.01
	β_1	3.59	0.38	<0.01	3.40	0.30	<0.01
	β_2	−0.35	0.05	<0.01	−0.15	0.01	<0.01
	Joint point	−0.22	0.19	NS	3.60	0.28	<0.01
	Time to max	5.16	0.22	<0.01	11.54	0.25	<0.01
	Value at max	12.07	0.44	<0.01	10.17	0.45	<0.01
100	*c*	1.00	0.00	<0.01	0.32	0.18	NS
	β_0	1.77	0.46	<0.01	−19.99	2.29	<0.01
	β_1	2.42	0.27	<0.01	3.62	0.35	<0.01
	β_2	−0.24	0.03	<0.01	−0.12	0.01	<0.01
	Joint point	−0.31	0.20	NS	7.43	0.23	<0.01
	Time to max	4.96	0.20	<0.01	15.08	0.20	<0.01
	Value at max	8.76	0.33	<0.01	7.67	0.36	<0.01

[a]Significance represents whether parameter estimate is different from zero. NS = not significant.
[b]Joint point = time (h) where exponential growth was initiated.
[c]Time to max = time (h) when maximal growth was achieved.
[d]Value at max = amount of growth (OD units) at the time when maximal growth was achieved.

TABLE 3
Selected List of Preplanned Contrasts of Estimated Parameters for Growth of *Staphylococcus aureus* Novel Strain with a Variety of Lipids

	Lipid				
Preplanned contrast[a]	Lauric acid	GML[b]	Capric acid	Linoleic acid	CLA[c]
			Pr > F[d]		
Joint point					
0 vs. 20 μg/mL	NS[e]	NS	NS	<0.01	<0.01
0 vs. 50 μg/mL	<0.01	<0.01	NS	<0.01	<0.01
0 vs. 100 μg/mL	<0.01	<0.01	0.02	<0.01	<0.01
20 vs. 50 μg/mL	<0.01	<0.01	<0.01	<0.01	<0.01
20 vs. 100 μg/mL	<0.01	<0.01	0.01	<0.01	<0.01
50 vs. 100 μg/mL	<0.01	<0.01	0.01	<0.01	<0.01
Max value					
0 vs. 20 μg/mL	<0.01	NS	NS	<0.01	NS
0 vs. 50 μg/mL	<0.01	<0.01	NS	<0.01	0.01
0 vs. 100 μg/mL	<0.01	<0.01	<0.01	0.02	<0.01
20 vs. 50 μg/mL	<0.01	<0.01	NS	NS	NS
20 vs. 100 μg/mL	<0.01	<0.01	<0.01	<0.01	NS
50 vs. 100 μg/mL	<0.01	<0.01	<0.01	0.01	NS

[a]Joint point = time when exponential growth was initiated; Max value = amount of growth (OD units) at the time when maximal growth was achieved.
[b]GML = glycerol monolaurate.
[c]CLA = *cis*-9, *trans*-11 conjugated linoleic acid.
[d]Pr > F = Probability of larger F.
[e]NS = not significant.

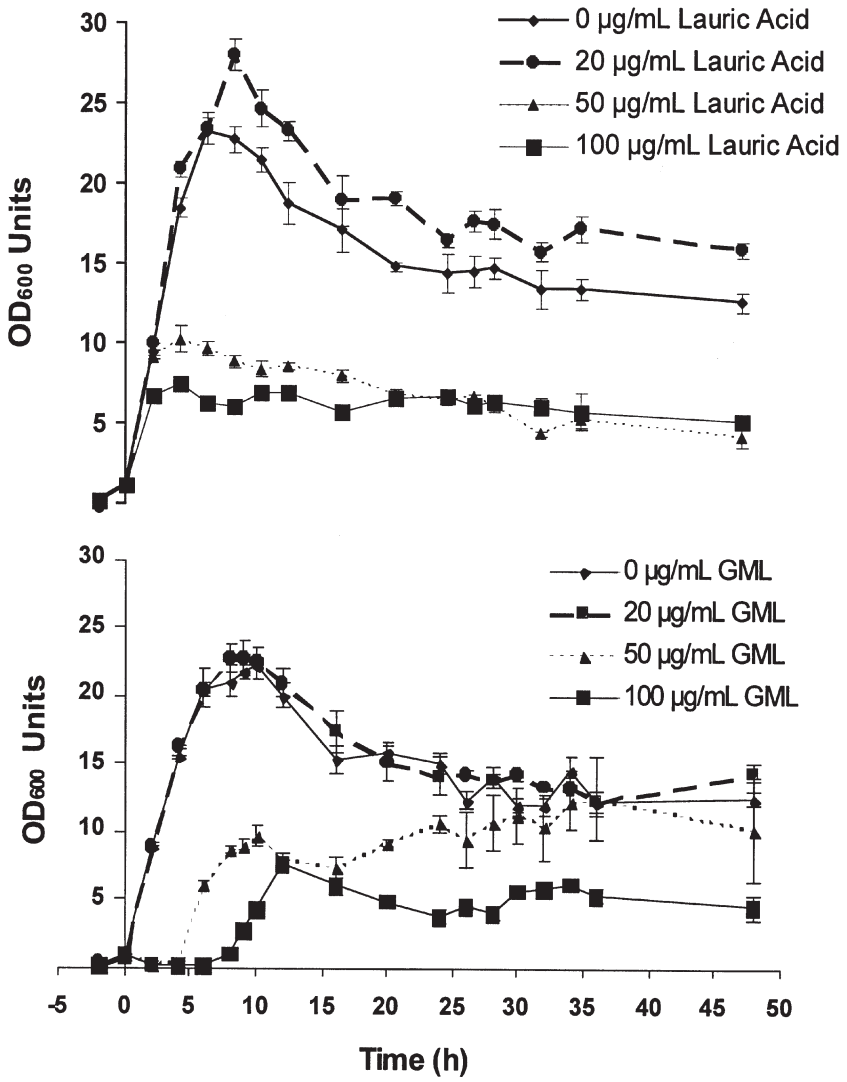

FIG. 2. Growth curves of *Staphylococcus aureus* Novel bacteria incubated with 0 (diamonds), 20 (circles), 50 (triangles), or 100 µg/mL (squares) of lauric acid (top panel) or glycerol monolaurate (bottom panel). Curves represent average of cultures performed in triplicate ± SE.

acid to the *S. aureus* resulted in increased membrane fluidity when measured by fluorescence polarimetry. This change in fluidity was detected as early as 30 s following treatment; however, significant killing occurred 45 min after treatment, indicating that oleic acid-induced killing was a multiple step process beginning with a change in fluidity of the membrane that may disrupt signal transduction cascades (33). Although proposed mechanisms (10) rely on models where the lipid interacts with the cell membrane to change permeability, more recent reports (34) indicate that some lipids including lauric acid and GML disrupt signal transduction cascades that inhibit the production of exoprotein virulence factors by *S. aureus* at concentrations lower than those required to affect growth. These observations support a multistep hypothesis to FA-induced inhibition.

FA release during infection. One indication of *S. aureus* infection is an increase in the concentration of FFA. This has been observed across a variety of infections in various animal models and in humans. Dye and Kapral (17) induced intraperitoneal abscesses in mice with *S. aureus* and found a substance produced in the abscess that was bactericidal, which was found in the lipid fraction, and further partitioning revealed it was the FFA portion. Once these FA were made into methyl esters, however, there was a loss of bactericidal activity (17). Further studies by Engler and Kapral (35) revealed a monoacylglycerol produced in intraperitoneal abscesses also had bactericidal activity. The 2-monoacylglycerol was the form originally produced; however, it spontaneously isomerized to a 1-monoacylglycerol. The FA moiety of the monoacylglycerol consisted of mostly palmitoleic acid and palmitic acid (35).

Patients with atopic dermatitis have recurring *S. aureus* skin infections and have a very different distribution of FA in their epidermis when compared to those without this condition (18). *In vitro* tests to evaluate the antimicrobial activity of human

TABLE 4
Parameter Estimates for Growth of *Staphylococcus aureus* Novel Supplemented with Capric Acid and Myristic Acid

	Capric acid				Myristic acid			
Parameter	Dose (μg/mL)	Parameter estimate	Standard error	*P* value[a]	Dose (μg/mL)	Parameter estimate	Standard error	*P* value
C	0	0.71	0.76	NS	0	0.55	1.94	NS
β_0		–2.61	1.36	NS		2.76	1.51	NS
β_1		6.61	0.44	<0.01		3.90	0.38	<0.01
β_2		–0.40	0.03	<0.01		–0.17	0.02	<0.01
Joint point[b]		0.51	0.22	0.03		–0.55	0.63	NS
Time to max[c]		8.21	0.14	<0.01		11.51	0.35	<0.01
Value at max[d]		25.24	0.88	<0.01		25.75	2.23	<0.01
C	20	0.74	0.76	NS	50	0.11	1.07	NS
β_0		–2.54	1.37	NS		1.45	0.84	NS
β_1		6.51	0.44	<0.01		3.91	0.21	<0.01
β_2		–0.36	0.03	<0.01		–0.16	0.01	<0.01
Joint point		0.52	0.22	0.02		–0.33	0.35	NS
Time to max		8.91	0.21	<0.01		12.22	0.23	<0.01
Value at max		27.21	0.88	<0.01		25.49	1.23	<0.01
C	50	0.58	0.79	NS	100	0.07	0.94	NS
β_0		–2.51	1.09	0.02		2.24	0.74	<0.01
β_1		5.72	0.28	<0.01		3.86	0.19	<0.01
β_2		–0.28	0.01	<0.01		–0.16	0.01	<0.01
Joint point		0.56	0.23	0.02		–0.55	0.31	NS
Time to max		10.07	0.13	<0.01		12.27	0.21	<0.01
Value at max		26.89	0.89	<0.01		26.05	1.08	<0.01
c	100	1.00	0.00	<0.01	200	1.00	<0.01	<0.01
β_0		1.74	0.85	0.04		3.95	1.04	<0.01
β_1		2.81	0.25	<0.01		1.35	0.20	<0.01
β_2		–0.11	0.02	<0.01		–0.02	<0.01	<0.01
Joint point		–0.26	0.31	NS		–2.1	0.95	0.03
Time to max		13.07	0.81	<0.01		26.91	3.79	<0.01
Value at max		21.14	0.57	<0.01		23.10	1.10	<0.01

[a]Significance represents whether parameter estimate is different from zero. NS = not significant.
[b]Joint point = time (h) where exponential growth was initiated.
[c]Time to max = time (h) when maximal growth was achieved.
[d]Value at max = amount of growth (OD units) at the time when maximal growth was achieved.

stratum corneum lipids against *S. aureus* have only been done with extracts from the skin of healthy individuals. However, the FFA fraction has been found to be the most effective class of lipid against growth of *S. aureus* (19). Further studies found lauric acid and linoleic acid to be the most active of these FFA, similar to the present study results (20).

The concentrations of FA tested in the present study were well within the normal range found in bovine and human milk. The profiles of bovine and human milk are slightly different, with bovine milk containing a higher content of short-chain saturated FA and human milk containing a higher concentration of unsaturated long-chain FA (36). Increased concentrations of FFA in milk are common during mastitis in cows (21). Increases of 50% to 100% have been detected in FFA content of milk from cows with mastitis (22–26). The concentrations of individual FFA during mastitis ranged from approximately 17 to 312 μg/mL with lauric acid and linoleic acid present at 50 and 56 μg/mL (an increase of 20% and 60% over normal concentrations), respectively (37). The increase in FFA during infection of the mammary gland was not constant among all FA (4.7% increase in capric acid vs. 77.2% increase in stearic acid), presumably due to the amount of that particular FA present in milk fat and their preferential position on the triglyceride molecule. Preference for butyric acid, caproic acid, and caprylic acid is at the *sn*-3 position, while lauric acid, myristic acid, and palmitic acid are typically located at the *sn*-2 position (36). However, lipases secreted by *S. aureus* have no positional specificity and hydrolyze FFA at all positions on the triglyceride equally (38). Nair *et al.* (39) tested the antibacterial effect of caprylic acid and monocaprylin and showed these lipids were able to kill five different mastitis pathogens in milk. However, the doses used were ~7,200 or ~14,000 μg/mL caprylic acid and ~5,500 or ~11,000 μg/mL monocaprylin, well above the range of those lipids present even in the triglyceride fraction of milk. The doses of FA used in the present study (20, 50, 100, and 200 μg/mL) are within the physiological range of those specific FA released during a mastitis infection.

Impact on host/pathogen interaction. FA at lower concen-

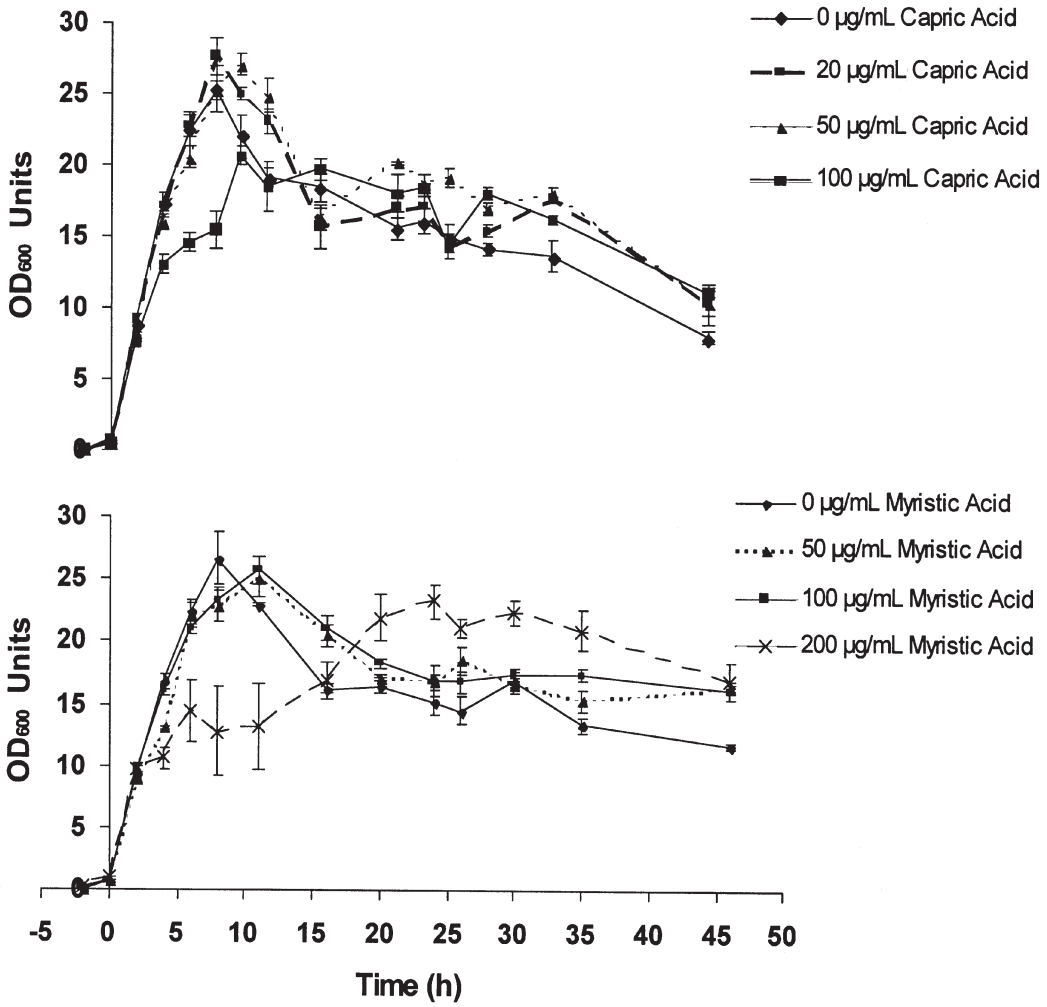

FIG. 3. Growth curves of *Staphylococcus aureus* Novel bacteria incubated with 0 (diamonds), 20 (circles), 50 (triangles), or 100 µg/mL (squares) of capric acid (top panel) or 0 (diamonds), 50 (triangles), 100 (squares), or 200 µg/mL (crosses) of myristic acid (bottom panel). Curves represent average of cultures performed in triplicate ± SE.

trations than those required for growth inhibition may also have other effects on the bacteria that are beneficial to the host. Lauric acid and GML have been more specifically analyzed in their ability to inhibit the growth and toxin production of *S. aureus*. Schlievert and coworkers (14) demonstrated the ability of GML to reduce the production of exotoxins by *S. aureus* at concentrations lower than those required for growth inhibition. The growth curves for the bovine mastitis *S. aureus* isolates followed a similar pattern to growth curves presented by Schlievert *et al.* (14) with human TSS isolates. Further work by Ruzin and Novick (15) found that free lauric acid at equimolar concentrations was just as effective as GML in blocking expression of protein A and TSS toxin-1 in *S. aureus.* Importantly, they found that active lipases secreted by *S. aureus* rapidly hydrolyze lauric acid from GML. This inhibition by lauric acid was at a dose of 20 µg/mL, a concentration 50-fold lower than that found in milk. Projan *et al.* (34) determined that GML inhibition of staphylococcal toxins occurred at the level of transcription. They also demonstrated that reduction of virulence factors was independent of the *agr* virulence regulatory system. Recently, GML was also shown to reduce three anthrax toxin components at the transcriptional level (40). We observed a difference in the pattern of growth inhibition between lauric acid (Fig. 2, top panel) and GML (Fig. 2, bottom panel). Lauric acid inhibited the maximum growth achieved by the bacteria whereas GML inhibited the growth of *S. aureus* through both a delay in the initiation of growth and a decrease in the maximum growth achieved. We speculate that this difference might be attributed to the amount of time it takes *S. aureus* to secrete active lipases able to cleave free lauric acid from the glycerol backbone of GML. Initially, GML inhibits the growth of *S. aureus* similarly to the polyunsaturated long-chain FA, creating a dose-dependent lag in the initiation of exponential growth. However, after 12 h, the pattern of growth is similar to that seen for lauric acid. Expression of lipases occurs normally during the postexponential phase of growth (41,42). However,

TABLE 5
Parameter Estimates for Growth of *Staphylococcus aureus* Novel Supplemented with *cis*-9, *trans*-11 Conjugated Linoleic Acid and Linoleic Acid

		cis-9, *trans*-11 CLA			Linoleic acid		
Dose (μg/mL)	Parameter	Parameter estimate	Standard error	*P* value[a]	Parameter estimate	Standard error	*P* value
0	*c*	0.13	0.64	NS	0.64	0.56	NS
	β_0	0.68	0.57	NS	−5.36	1.43	<0.01
	β_1	5.43	0.27	<0.01	7.53	0.47	<0.01
	β_2	−0.32	0.02	<0.01	−0.45	0.03	<0.01
	Joint point[b]	−0.03	0.20	NS	0.84	0.18	<0.01
	Time to max[c]	8.29	0.27	<0.01	8.28	0.14	<0.01
	Value at max[d]	23.35	0.70	<0.01	26.52	0.74	<0.01
20	*c*	0.47	0.94	NS	0.99	0.56	NS
	β_0	−14.28	3.15	<0.01	−38.55	5.34	<0.01
	β_1	5.05	0.58	<0.01	10.58	1.03	<0.01
	β_2	−0.16	0.02	<0.01	−0.40	0.05	<0.01
	Joint point	3.26	0.46	<0.01	4.51	0.26	<0.01
	Time to max	15.23	0.57	<0.01	13.14	0.30	<0.01
	Value at max	24.71	1.28	<0.01	31.98	0.92	<0.01
50	*c*	0.75	0.28	0.01	0.98	0.29	<0.01
	β_0	−51.09	4.30	<0.01	−52.88	4.35	<0.01
	β_1	6.19	0.43	<0.01	8.12	0.55	<0.01
	β_2	−0.12	0.01	<0.01	−0.20	0.02	<0.01
	Joint point	10.71	0.28	<0.01	8.35	0.21	<0.01
	Time to max	24.52	0.34	<0.01	20.26	0.31	<0.01
	Value at max	25.59	0.51	<0.01	30.42	0.55	<0.01
100	*c*	0.92	0.38	0.02	0.87	0.24	<0.01
	β_0	−98.05	12.64	<0.01	−139.24	10.14	<0.01
	β_1	8.83	1.13	<0.01	13.19	0.95	<0.01
	β_2	−0.15	0.02	<0.01	−0.26	0.02	<0.01
	Joint point	15.46	0.33	<0.01	15.16	0.17	<0.01
	Time to max	28.08	0.86	<0.01	25.29	0.31	<0.01
	Value at max	26.90	0.73	<0.01	28.52	0.46	<0.01

[a]Significance represents whether parameter estimate is different from zero. NS = not significant.
[b]Joint point = time (h) where exponential growth was initiated.
[c]Time to max = time (h) when maximal growth was achieved.
[d]Value at max = amount of growth (OD units) at the time when maximal growth was achieved.

some exoproteins including lipases may have individual specific regulatory mechanisms that may be induced by their substrates (41). The effect on *S. aureus* toxin production has been observed in lauric acid and GML; however, we are unaware of any reports in which these effects have been observed with polyunsaturated long-chain FA.

These results indicate that release of FFA or monoacylglycerols may be a mechanism to inhibit growth of *S. aureus* during an infection. At subinhibitory concentrations, these FA may also help to minimize damage to host tissues due to virulence factors such as toxins secreted by *S. aureus.*

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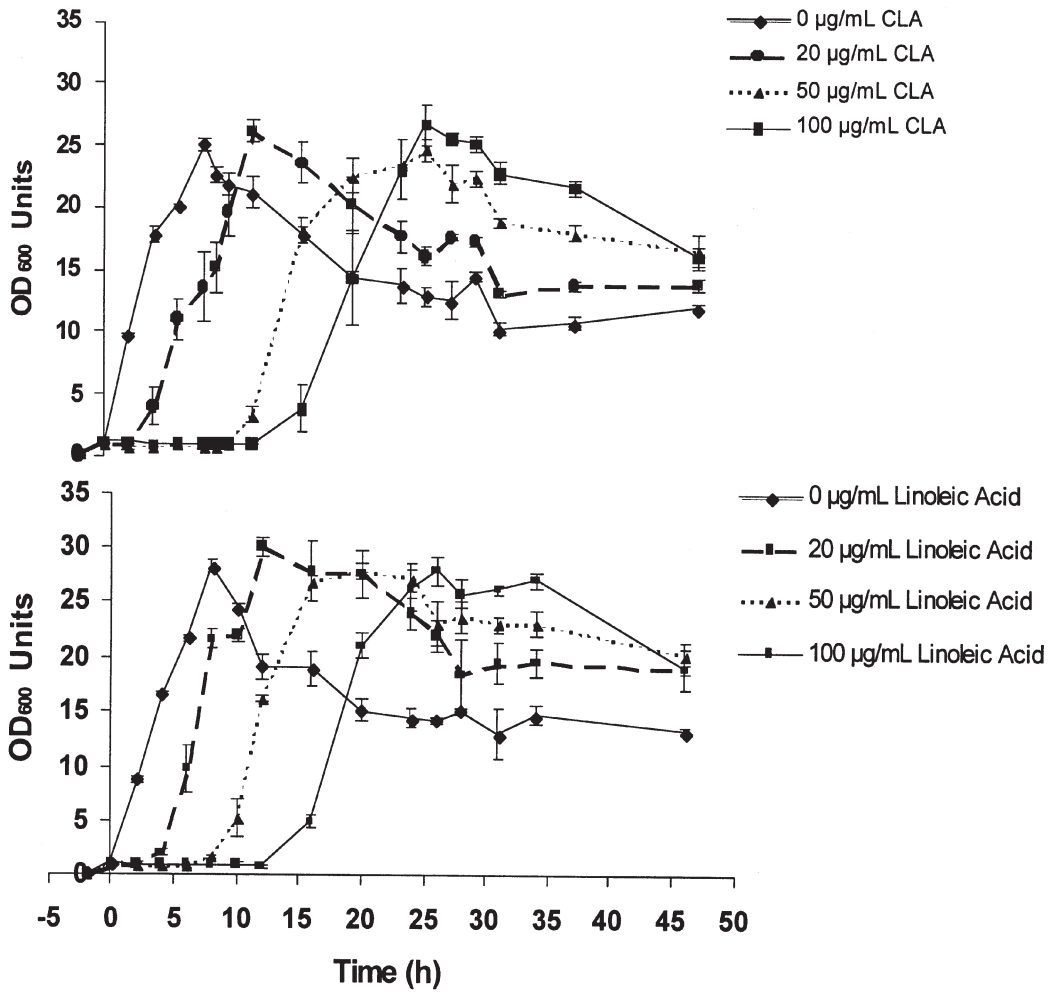

FIG. 4. Growth curves of *Staphylococcus aureus* Novel bacteria incubated with 0 (diamonds), 20 (circles), 50 (triangles), or 100 µg/mL (squares) of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) (top panel) or linoleic acid (bottom panel). Curves represent average of cultures performed in triplicate ± SE.

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