Temperature-Related Variations in the Lipid Composition of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Strains

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Growth of methicillin-susceptible and -resistant strains of *Staphylococcus aureus* at 28 and 37°C led to changes in the relative amounts of phospholipids recovered. The major phospholipids present in these strains were phosphatidylglycerol, lysophosphatidylglycerol, and diphosphatidylglycerol. Phosphatidylglycerol tended to occur in lower amounts in the resistant strains, but it was the major phospholipid in both methicillin-susceptible and -resistant *S. aureus* strains. Growth patterns and total lipid concentrations were similar in all strains.

It has long been recognized that multiple factors influence the degree and extent of methicillin resistance within naturally occurring strains of methicillin-resistant *Staphylococcus aureus* (7). Rozgonyi et al. (6) found that methicillin influenced the phospholipid composition in both methicillin-susceptible and -resistant strains of *S. aureus*. They suggested that the antibiotic caused a partial inhibition of phospholipid synthesis, thereby affecting the composition of the cell membrane.

In view of the foregoing, an objective of the present investigation was to compare the growth and the total lipid and phospholipid composition of methicillin-susceptible and -resistant strains of *S. aureus*. The strains were grown at 28 and 37°C to determine whether temperature contributes to changes in the lipid composition of either type.

Four strains of *S. aureus* obtained from Analytab Products, Inc., Plainview, N.Y. were used. The strains and their susceptibility to methicillin were as follows: 175-78R and 177-78R, resistant; 184-78S and 185-78S, susceptible. Stock cultures were maintained on heart infusion agar (Difco Laboratories, Detroit, Mich.). The methicillin susceptibility of the *S. aureus* strains was determined throughout the course of this study by using Mueller Hinton agar (Difco) and a standardized disk method (2). Resistance was defined as a zone diameter of less than 9 cm, and susceptibility as a zone greater than 14 cm. None of the strains displayed any significant change in susceptibility throughout this study.

Each strain was grown aerobically in heart infusion broth (Difco) at both 28 and 37°C. Cell populations were measured by viability counts, which were performed in duplicate at frequent intervals over a period of 72 h. All strains showed good growth, with maximal populations occurring between 15 and 18 h of incubation at the indicated temperature (Table 1). In general, the viable populations obtained at 28°C were similar to those obtained at 37°C.

Total lipid extractions were performed on lyophilized cells harvested after 13 h of incubation, using the procedure of Huston and Albro (4). Lipid extracts were purified on Sephadex G-25 (8). Phospholipids were isolated from the purified lipid extract by thin-layer chromatography on Silica Gel H, using as a solvent system chloroform-methanol-water (65:25:4, vol/vol/vol) (5). Iodine vapors were used to locate the phospholipids, which were recovered and quantitated by phosphorus content (1).

The amount of total lipid obtained from the *S. aureus* strains varied from 2.6 to 3.1%, based on the dry weight of cells (Table 2). The lowest and highest amounts were found in methicillin-susceptible strains. Overall, however, the lipid content of the strains was remarkably similar. In addition, it was observed consistently that total lipid yields for a given strain were always slightly higher at 28°C. The increases ranged from 3.8 to 11.5%.

The major phospholipids present in the strains of *S. aureus* examined were phosphatidylglycerol (PG), lysophosphatidylglycerol (LPG), and diphosphatidylglycerol (DPG). The relative amounts of each phospholipid isolated from each strain grown at 28 and 37°C are shown in Table 3.

The methicillin-resistant strains, *S. aureus*

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TABLE 1. Relationship of temperature to maximal growth of methicillin-sensitive and -resistant strains of *S. aureus*

Strain no.	Growth ^a (CFU/ml) at:	
	28°C	37°C
175-78R	2.5×10^8 (16)	2.3×10^8 (16)
177-78R	9.8×10^7 (16)	2.3×10^8 (16)
184-78S	9.0×10^7 (16)	2.5×10^8 (15)
185-78S	1.7×10^8 (16)	2.4×10^8 (18)

^a Numbers in parentheses indicate the time (in hours) at which maximal growth was attained.

175-78R and 177-78R, displayed a similar quantitative distribution of the major phospholipids. PG predominated at both temperatures, although lesser amounts were recovered from cells grown at 28°C. In strain 175-78R, for example, the decrease in the amount of PG recovered at 28°C compared with that at 37°C amounted to 22%. In contrast to PG, yields of DPG increased with decreasing temperature. Increases of 71 and 117% for strains 175-78R and 177-78R, respectively, were noted. LPG levels remained comparatively constant in all strains at both temperatures.

The methicillin-susceptible strains, *S. aureus* 184-78S and 185-78S, also showed similarities in the relative amounts of phospholipids recovered. In each case, PG occurred in the greatest quantity, followed by LPG and DPG, respectively. Strains 184-78S and 185-78S showed decreases in PG of approximately 12% when the temperature was shifted to 28°C. As in the methicillin-resistant strains, the DPG concentration increased significantly when the temperature was lowered to 28°C. Strains 184-78S and 185-78S, for example, showed increases of 75 and 104%, respectively. As was the case with the methicillin-resistant bacteria, the least dra-

TABLE 3. Effect of temperature on the phospholipid composition of methicillin-sensitive and -resistant strains of *S. aureus*

Strain no.	Growth temp (°C)	Amt (% of total phospholipids) of:		
		LPG	PG	DPG
175-78R	28	33.8	37.9	28.2
	37	34.9	48.6	16.5
177-78R	28	34.7	40.6	24.7
	37	37.4	51.2	11.4
184-78S	28	26.6	57.9	15.6
	37	25.0	66.1	8.9
185-78S	28	23.2	62.7	14.1
	37	21.7	71.4	6.9

matic temperature-induced changes were in the LPG levels.

A difference in the phospholipid composition between methicillin-resistant and -susceptible strains was the relatively higher levels of PG in the latter. The susceptible strains at 37°C, for example, had an average of 69% PG, compared with 50% for the resistant strains. At 28°C, the values were 60% for the susceptible strains and 39% for the resistant ones. As would be expected, the higher levels of PG in the susceptible strains were accompanied by relatively lower concentrations of both LPG and DPG than were found in the resistant strains.

The work reported here shows that growth temperature significantly influenced the levels of phospholipids isolated from both methicillin-susceptible and -resistant strains of *S. aureus*. In several cases the alterations in phospholipid composition amounted to over 100%. These observations are of particular interest since the total lipid fractions from all strains examined were relatively similar in quantity. Our findings do not coincide with a previous report that cultivation of *S. aureus* and other gram-positive bacteria under conditions leading to an increase in cellular lipids caused a corresponding increase in their resistance to methicillin and other penicillin antibiotics (3). Under our conditions, significant increases in cellular lipids did not occur in either methicillin-susceptible or -resistant strains in response to temperature alterations. Consequently, the opportunity to relate increases in lipid yields to methicillin resistance did not develop. The present investigation does show that PG levels tended to be lower in resistant than in susceptible strains. Thus, variations in the relative amounts of the phospholipids present may correlate with the degree of

TABLE 2. Effect of temperature on total lipid composition of methicillin-sensitive and -resistant strains of *S. aureus*

Strain no.	Growth temp (°C)	Total lipids (% of dry wt)
175-78R	28	3.0
	37	2.7
177-78R	28	2.9
	37	2.6
184-78S	28	2.7
	37	2.6
185-78S	28	3.1
	37	2.8

susceptibility to methicillin. This observation is in agreement with that of Rozgonyi et al. (6), who reported that in methicillin-free medium, PG levels were higher in *S. aureus* strains that were susceptible to the antibiotic.

The process by which microorganisms become resistant to methicillin is not entirely clear, and a variety of factors apparently influence the degree and extent of this resistance (7). If, as has been suggested (6), the phospholipid composition contributes to this process, our data strongly indicate that careful attention must be paid to the growth temperature used. Temperature alterations caused moderate to severe changes in the relative proportions of individual phospholipids contained in both methicillin-susceptible and -resistant *S. aureus* strains. If any definitive conclusions are to be drawn about the relationship between methicillin resistance mechanisms and cellular phospholipids, the growth temperature is a variable that must be rigidly controlled.

LITERATURE CITED

1. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**:466-468.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized disc method. *Am. J. Clin. Pathol.* **45**:493-496.
3. Hugo, W. B., and R. J. Stretton. 1966. The role of cellular lipid in the resistance of gram-positive bacteria to penicillins. *J. Gen. Microbiol.* **42**:133-138.
4. Huston, C. K., and P. W. Albro. 1964. Lipids of *Sarcina lutea*. I. Fatty acid composition of the extractable lipids. *J. Bacteriol.* **88**:425-432.
5. Rouser, G., A. N. Siakotos, and S. Fleischer. 1966. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids* **1**:85-86.
6. Rozgonyi, F., L. Vácz, P. Sebessy Gönczy, and I. Rédei. 1973. Phospholipid content of staphylococci sensitive and resistant to methicillin. *Contrib. Microbiol. Immunol.* **1**:172-179.
7. Sabath, L. D., and S. J. Wallace. 1971. Factors influencing methicillin resistance in staphylococci. *Ann. N.Y. Acad. Sci.* **182**:258-266.
8. Siakotos, A. N., and G. Rouser. 1965. Analytical separation of nonlipid water soluble substances and gangliosides from other lipids by dextran gel column chromatography. *J. Am. Oil Chem. Soc.* **42**:913-919.