

## TEMPERATURE INDUCED SHIFTS IN THE FATTY ACID PROFILE OF *STAPHYLOCOCCUS AUREUS* WRRC B124<sup>1</sup>

BOBBY L. BOWLES, THOMAS A. FOGLIA<sup>2</sup> and VIJAY K. JUNEJA

*U.S. Department of Agriculture, Agricultural Research Service  
Eastern Regional Research Center  
600 East Mermaid Lane  
Philadelphia, PA 19118*

Received for Publication January 24, 1996

### ABSTRACT

*Variation in the fatty acid profile of Staphylococcus aureus WRRC B124 grown at varying temperatures was determined. The range of incubation temperatures tested induced both qualitative and quantitative differences in S. aureus fatty acid profile. While branched chained saturated fatty acids were the predominate species at 37C, C18:1 and C16:1 monounsaturated fatty acids were predominant at lower temperatures (12 and 19C). Iso-branched C14:0, C16:0 and C18:0 saturated fatty acids were expressed exclusively at 37C and several C17:0 and C20:0 fatty acids were suppressed at 12C. Results demonstrated that S. aureus had altered fatty acid profile in response to changes in growth temperature.*

### INTRODUCTION

Fatty acid composition of bacteria depends on the environmental conditions present during growth, i.e., pH, atmosphere, temperature, and growth medium (Miller and Berger 1985). It is well-known that microorganisms alter their membrane lipid composition in response to changes in temperature (Cronan and Gelman 1975). The changes in fatty acid profiles are consistent with the hypothesis that cells adapt their membrane lipids to compensate for the alteration in the temperature of incubation. Sinensky (1974) suggested that an appropriate level of membrane fluidity must be maintained by bacteria in response to temperature

<sup>1</sup>Reference of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

<sup>2</sup>Correspondence to: T.A. Fologia, Eastern Regional Research Center, ARS, USDA, 600 East Mermaid Lane, Philadelphia, PA 19118, (215) 233-6480.

change. Homeoviscous adaptation has been used to describe this mechanism by which organisms attempt to avoid excessive order or fluidization of membrane lipids. The change is brought about substantially by alteration of the relative abundance of saturated and unsaturated membrane fatty acids (Sinensky 1974). Jackson and Cronan (1978), however, indicated that the measured average membrane fluidity level of the bacterial membrane may not be as important as the need for a minimal amount of fluid lipid domain within the membrane. High melting point fatty acids (saturated, long and straight chain fatty acids) decrease membrane fluidity (McElhaney 1976), whereas low melting point fatty acids (i.e., unsaturated and branched chain fatty acids) increase membrane fluidity (Welker 1976). In general, as growth temperature of bacteria is increased, membrane lipids contain increasing amounts of saturated fatty acids that serve to maintain an appropriate level of membrane fluidity (Gounot 1991). Therefore, changes in incubation conditions may confound identification of bacterial species using fatty acid profiles.

As such, we investigated the effect of growth temperatures on the fatty acid transitions of *S. aureus*. The purpose of this study was to determine whether the fatty acid profiles of *S. aureus* was altered in a systematic way in response to changes in growth temperature.

## MATERIALS AND METHODS

### Bacterial Strain

Coagulase positive *Staphylococcus aureus* WRRC B124 was obtained from the USDA/ARS Midwest Area Regional Research Center (Peoria, Illinois). Species identification was based on catalase activity, coagulase or thermonuclease production, and anaerobic utilization of glucose and mannitol (Bennett 1984). Brain heart infusion broth (BHI - pH 7.4 ± 0.2) or agar (BHIA - pH 7.4 ± 0.2) was the test medium used throughout the study. Approximately 0.1 ml of a *S. aureus* WRRC B124 stock culture was transferred to 150 ml trypticizing flasks containing 9.9 ml BHI broth (pH 7.4 ± 0.2) and incubated aerobically 24 ± 2 h at 37C.

### Fatty Acid Analysis — Culture Preparation and Incubation

Duplicate sets of trypticizing flasks (Bellco; 150) containing 25 ml BHI broth were inoculated with 0.1 ml of a 24 h *S. aureus* culture, and then incubated at 37, 19 or 12C on a model G-26 New Brunswick shaker (120 rpm). Cells were incubated to an absorbance at 600 nm of 0.6 O.D.

### Harvesting and Concentration of Cells

*S. aureus* cells were harvested by three successive centrifugations at  $17,310 \times g$  for 10 min at 2–4°C with sterile distilled- $H_2O$  washes between centrifugations. The cell pellets were resuspended in sterile distilled- $H_2O$ , and concentrated by lyophilization in a Labconco Freeze dryer (Model 77530; Labconco Corporation, Kansas City, MO). Lyophilized samples were stored at 4°C prior to analyses. A duplicate set of lyophilized samples was then subjected to lipid extractions, methanolysis and fatty acid analysis for each incubation temperature tested.

### Lipid Extraction and Methanolysis

Lipids present in dried biomass were extracted and converted to methyl esters using a modification of the methanolysis procedure described by Minnikin *et al.* (1980). Approximately 20–40 mg of lyophilized *S. aureus* WRRRC B124 cells were transferred to a 10-ml glass centrifuge tube with a polytetrafluorethylene screw cap (Thomas Scientific, Swedesboro, NJ) and 3 ml of dry methanol/toluene/trifluoromethanesulfonic acid (30:15:1) mixture added. The reaction mixture was heated at 60°C for 16–18 h, cooled to room temperature, 2 ml of hexane added, and the mixture vortexed for 1 min. The phases were allowed to separate and the upper hexane layer was transferred to a small column of basic alumina (75 mg, 150 mesh, 58A°) (Aldrich Chemical, St. Louis, MO) prepared in a jumbo glass-wool plugged Pasteur pipette, previously washed with 2–3 ml of methylene chloride. The hexane eluant was collected in a 1-dram vial. The reaction mixture was reextracted with hexane (1 ml) and the upper layer pipetted onto the column. The combined eluants were evaporated under nitrogen at 20°C, the residue weighed for percent lipid determination (w/w), and then dissolved in isooctane (0.10 ml) containing methyl heneicosanoate (C21:0, 1.08 mg/ml) as an internal standard for gas-liquid chromatographic analysis (GLC).

### Fatty Acid Analyses

Fatty acid methyl esters (FAME) were analyzed on a Hewlett-Packard (Avondale, PA) Model 5890 gas chromatograph equipped with a split capillary injector and a flame ionization detector. Separations were obtained using a fused silica capillary column, 60 m  $\times$  0.25 I.D., coated with SP-2340 (Supelco, Bellefonte, PA). The carrier gas was He (linear velocity methane, 22.9 cc sec<sup>-1</sup>) at a split ratio of 60:1. The following oven temperature program was used: initial temperature 140°C; then 0.5°C min<sup>-1</sup> to 150°C then 2°C min<sup>-1</sup> to 200°C; then hold for 20 min. Methyl heneicosanoate (C21:0) served as internal standard. Signal

analysis was accomplished by routing the detector output to a Hewlett-Packard Model 3396A integrator and a Hewlett-Packard Model 9122C mass storage unit for subsequent statistical analysis. FAME assignments were made by comparison with standards (NuCheck Prep, Elysian, MN) or from plots of log retention time versus carbon number (Slover and Lanza 1979). Chain length assignment for FAME with unspecified double bond positions was accomplished by hydrogenation of the original methyl ester sample ( $H_2/PtO$ ) in a Parr (Moline, IL) hydrogenator and reanalyzing the sample on GLC.

## RESULTS AND DISCUSSION

Fatty acid compositions of *S. aureus* was surveyed for alterations in response to changes in growth temperature. This variability could be important since gas-liquid chromatography of fatty acid methyl esters has been used to identify *S. aureus*. Quantitative and qualitative temperature dependent differences were observed in the types of *S. aureus* fatty acid composition (Table 1). The majority of *S. aureus* WRRC B124 fatty acids were saturated at 37C (96.23%), whereas unsaturated fatty acids predominated at 19 and 12C (Fig. 1). The relative amounts of mono- and polyunsaturated fatty acids were similar at 37C, whereas monounsaturated fatty acids were present in amounts of 60 and 100 times greater than those of polyunsaturated fatty acids at 19 or 12C, respectively. At 37C approximately 73% of the saturated fatty acids were branched; with 26% being iso-branched, e.g., i-C15:0 (13-methyltetradecanoic acid), and 47% being anteiso-branched, e.g., ai-C15:0 (12-methyltetradecanoic acid) (Fig. 2). The amounts of branched chain fatty acids were significantly lower, however, at 19 (14.46%) and at 12C (3.76%).

Several *S. aureus* fatty acids were suppressed or expressed only at specific incubation temperatures (Table 1). Fatty acids with i-C14:0, i-C16:0, i-C18:0 and i-C19:0 acyl groups were expressed exclusively at 37C, while those containing C14:1, C15:0, C16:1, C16:1n7 and C18:1n9t acyl groups were suppressed. At 12C, fatty acids with ai-C17:0, C17:0, i-C20:0 and C20:0 acyl groups were suppressed. Temperature induced qualitative differences in fatty acid profiles were investigated to differentiate staphylococci from *Micrococcus* spp. and other Gram-positive bacteria (Schleifer and Kroppenstedt 1990). Synthesis of *S. aureus* specific *n*-eicosanoic acid (C20:0) was suppressed at 12C, and quantitatively reduced at 19C. The percent content of octadecanoic acid (C18:0), another saturated fatty acid that distinguishes staphylococci from other Gram-positive bacteria, was reduced at temperatures above or below 37C (Schleifer and Kroppenstedt 1990).

TABLE 1.  
TEMPERATURE INDUCED TRANSITIONS IN *S. AUREUS* WRRC B124 WHOLE CELL  
METHANOLYSATE FATTY ACIDS

Acyl Group <sup>a</sup>	Fatty Acid Content (%) <sup>b</sup> at Incubation Temperature ( C)		
	37	19	12
i-C14:0	0.74	- <sup>c</sup>	-
C14:0	0.28	6.53	7.05
C14:1	-	1.32	2.35
C14:1	-	0.67	0.76
i-C15:0	9.10	0.62	0.09
ai-C15:0	29.70	1.51	0.32
C15:0	-	0.14	0.12
i-C16:0	1.62	-	-
C16:0	2.28	16.15	13.13
C16:1	-	1.34	1.48
C16:1 $\pi$ 7	-	10.93	10.67
i-C17:0	7.39	1.03	0.10
ai-C17:0	10.36	0.62	-
C17:0	0.46	0.20	-
i-C18:0	1.58	-	-
C18:0	10.75	4.85	2.87
C18:1 $\pi$ 9t	-	0.55	0.42
C18:1 $\pi$ 9c	0.45	44.12	53.59
i-C19:0	4.62	-	-
ai-C19:0	4.90	0.33	0.40
C19:0	1.87	0.17	0.13
C18:2 $\pi$ 9,12	0.19	0.43	0.19
i-C20:0	0.45	0.67	-
ai-C20:0	0.13	-	-
C20:0	10.00	0.23	-
C18:3 $\pi$ 9,12,15	0.33	0.60	0.58
C20:1	0.13	2.32	3.33
Unknown	1.40	2.73	2.55

<sup>a</sup>Acyl group: i-iso; ai-anteiso; *n*-location of double bond; t-trans; c-cis.

<sup>b</sup>Area weight %,  $\pm$  0.1%

<sup>c</sup>Species absent or below detection limit.

The distribution of fatty acid carbon chain lengths varied with the incubation temperature (Fig. 3). At 37C, C15 (38.8%), C17 (18.21%), C18 (12.78%) and C19 (11.39%) fatty acids were the predominate species; C16 (28.42%) and C18 (49.52%) predominated at 19C, and C16 (25.28%) and C18 (56.88%) predominated at 12C.

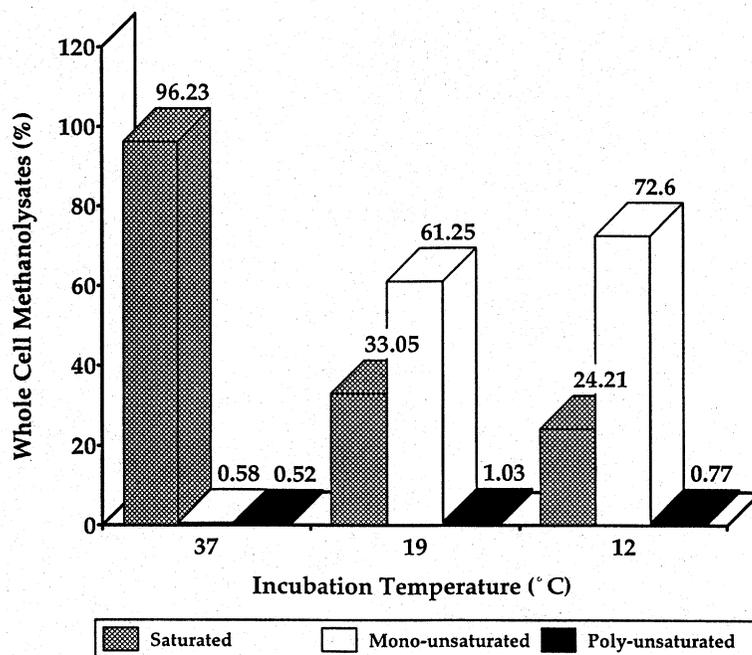


FIG. 1. TEMPERATURE INDUCED TRANSITIONS IN *S. AUREUS* WRRc B124 SATURATED AND UNSATURATED FATTY ACIDS IN BRAIN HEART INFUSION BROTH

Temperature-induced fatty acid alterations have been studied extensively (McGarrity and Armstrong 1975; Marr and Ingraham 1962; Khuller and Goldfine 1974; Miller 1985) and have been attributed to adaptations. Khuller and Goldfine (1974) generalized the changes in fatty acid composition in bacteria in response to the temperature of environment and reported that when growth temperature is lowered, fatty acid composition is modified in the direction of lower average melting points. In certain Gram-positive bacteria there are increased amounts of lower-melting branched-chain fatty acids at the expense of the higher melting branched and straight-chain fatty acids at lower growth temperatures (Weerkamp and Heinen 1972).

In the present study, *S. aureus* unsaturated fatty acids were significantly higher at temperatures above and below the 37°C optimum growth temperature. Mono-unsaturated fatty acids were the predominate species at 19 or 12°C and synthesis of polyunsaturated fatty acids was highest at 12°C. Post and Davidson (1986) reported that the ratio of saturated to unsaturated fatty acids of several pathogenic

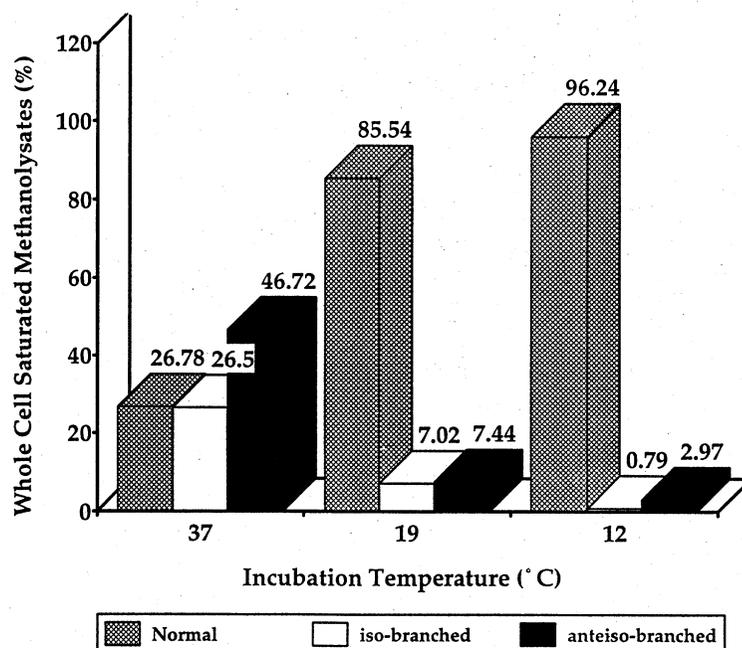


FIG. 2. DISTRIBUTION OF *S. AUREUS* WRRc B124 SATURATED FATTY ACID METHYL ESTERS AT VARIOUS INCUBATION TEMPERATURES  
 Total whole cell saturated fatty acids were 96.23, 33.05 and 24.21%, respectively, at 37, 19 and 12C.

and spoilage bacteria, including *S. aureus*, did not account for the differences in susceptibility to butylated hydroxyanisole (BHA) susceptibility among the test organisms. Different substrates were used for the organisms tested, however, and the cultures were incubated at temperatures ranging from 26 to 37C.

The fatty acid profile found at 37C in BHI broth was comparable to those reported by other investigators (Durham and Kloos 1978; O'Donnell *et al.* 1985). At incubation temperatures above or below the 37C optimum, > 36% were long chain fatty acids, with appreciable amounts of 17 carbon-length fatty acids observed only at 37C. The majority of fatty acids synthesized at 19 or 12C were C18:0 or C16:0 monounsaturated fatty acids, with significant quantities of normal chain saturated fatty acids produced.

In conclusion, both quantitative and qualitative modulations in Fatty acid profile of *S. aureus* WRRc B124 were found in response to different incubation temperatures. Our results indicate that if gas-liquid chromatography is to be used to identify *S. aureus* strains, incubation conditions must be controlled.

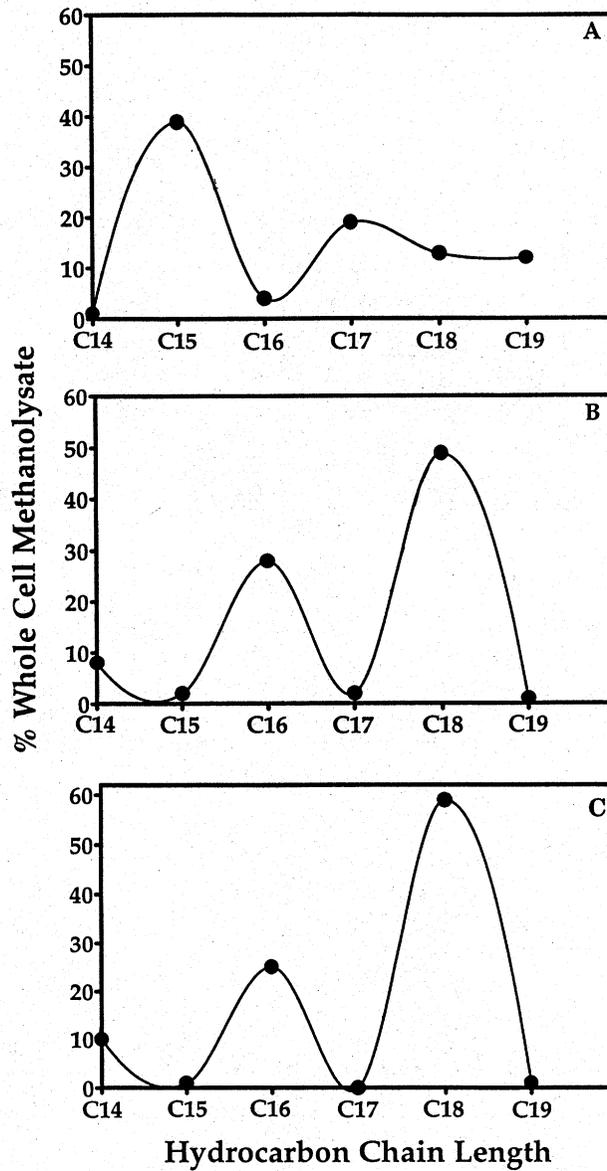


FIG. 3. EFFECT OF TEMPERATURE ON THE PREDOMINATING HYDRO-CARBON CHAIN LENGTH AND DISTRIBUTION OF *S. AUREUS* WRC B124 WHOLE CELL METHANOLYSATE FATTY ACIDS  
 A, B, and C represent incubation temperatures of 37, 19 and 12C, respectively.

## ACKNOWLEDGMENTS

The technical assistance of Lenier W. Tucker, Aaron C. Williams and Solomon K. Sacikety is greatly appreciated.

## REFERENCES

- BENNETT, R.W. 1984. *Staphylococcus aureus*. In *Bacteriological Analytical Manual of the Division of Microbiology* (6th Ed.), Ch. 14, pp. 1-5, Center for Food Safety & Applied Nutrition, U.S. Food and Drug Administration, AOAC, Arlington, Virginia.
- CRONAN, J.E., Jr. and GELMAN, E.P. 1975. Physical properties of membrane lipids: biological relevance and regulation. *Bacteriol. Rev.* 39, 232-256.
- DURHAM, D.R. and KLOOS, W.E. 1978. Comparative study of the total cellular fatty acids of *Staphylococcus* species of human origin. *Int. J. System. Bacteriol.* 28(2), 223-228.
- GOUNOT, A. 1991. Bacterial life at low temperature: physiological aspects and biotechnological implications. A review. *J. Appl. Bacteriol.* 71, 386-397.
- JACKSON, M.B. and CRONAN, J.E. 1978. An estimate of the minimum amount of fluid lipid required for the growth of *Escherichia coli*. *Biochim. Biophys. Acta* 512, 472-479.
- KHULLAR, G.K. and GOLDFINE, H. 1974. Phospholipids of *Clostridium butyricum*. V. Effects of growth temperature on fatty acid, alk-1-enyl ether group, and phospholipid composition. *J. Lipid Research.* 15, 500-507.
- MARR, A.G. and INGRAHAM, J.L. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* 84, 1260-1267.
- McELHANEY, R.N. 1976. In *Extreme Environments: Mechanisms of Microbial Adaptation*, (M.R. Henrich, ed.) pp. 255-281. Academic Press, New York.
- McGARRITY, J.T. and ARMSTRONG, J.B. 1975. The effect of temperature on phospholipid and fatty acid composition in *E. coli* K-12. *Biochem Biophys. Acta.* 398, 258.
- MILLER, K.J. 1985. Effects of temperature and sodium chloride concentration on the phospholipid and fatty acid composition of a halotolerant *Planococcus* sp. *J. Bacteriol.* 162, 263-270.
- MILLER, L. and BERGER, T. 1985. Bacterial identification by gas chromatography of whole cell fatty acids. Hewlett-Packard Application Note 228. Hewlett-Packard Co., Avondale, PA.
- MINNIKIN, D.E., HUTCHINSON, I.G. and CALDICUTT, A.B. 1980. Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J. Chromatog.* 188, 221-233.

- O'DONNELL, A.G., NAHAIE, M.R., GOODFELLOW, M., MINNIKIN, D.E. and HÁJEK, V. 1985. Numerical analysis of fatty acid profiles in the identification of staphylococci. *J. Gen. Microbiol.* *131*, 2023-2033.
- POST, L.S. and DAVIDSON, P.M. 1986. Lethal effect of butylated hydroxyanisole as related to bacterial fatty acid composition. *Appl. Environ. Microbiol.* *52*, 214-216.
- SCHLEIFER, K.H. and KROPPESTEDT, R.M. 1990. Chemical and molecular classification of staphylococci. *J. Appl. Bacteriol.* *69*, 9S-24S.
- SINENSKY, M. 1974. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* *71*, 522-525.
- SLOVER, H.T. and LANZA, E. 1979. Quantitative analysis of food fatty acids by capillary gas chromatography. *J. Amer. Oil Chem. Soc.* *56*, 933-943.
- WEERKAMP, A. and HEINEN, W. 1972. Effect of temperature on the fatty acid composition of the extreme thermophiles, *Bacillus caldolyticus* and *Bacillus caldotenax*. *J. Bacteriol.* *109*, 443-446.
- WELKER, N.M. 1976. In *Extreme Environments: Mechanisms of Microbial Adaptation*. (M.R. Heinrich, ed.) pp. 229-254, Academic Press, New York.