

## INFLUENCE OF TEMPERATURE ON THE FATTY ACID PROFILE OF *LISTERIA MONOCYTOGENES*

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### ABSTRACT

*Variation in the fatty acid profile of two Listeria monocytogenes strains grown at varying temperatures was determined. The fatty acid profiles varied greatly at different temperatures. General decreases in relative percentages of branched and medium chain (up to C16:0) fatty acids and variable changes in long chain fatty acids were found with increasing growth temperature. Individual fatty acid percentages between strains were variable. The relative percentages of unknown long chain fatty acids, detected in both strains at various temperatures, were greatest in Scott A (7.07%) and ATCC 19114 (13.15%) at 35C. Results demonstrated that L. monocytogenes had altered fatty acid profile in response to changes in growth temperature.*

### INTRODUCTION

A large amount of data exists concerning bacterial fatty acid composition. Bacteria often contain unique fatty acids, including  $\beta$ -hydroxy-, cyclopropane, and branched-chain fatty acids. Certain types of fatty acids predominate in a given taxon and may be of importance in identifying bacteria using gas-liquid chromatography (Moss *et al.* 1974).

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Miller and Berger (1985) found that fatty acid composition of bacteria depends on the environmental conditions present during growth, i.e., pH, atmosphere, temperature, and growth medium. It is well-known that microorganisms alter their membrane lipid composition in response to changes in temperature (Cronan and Gelman 1975). Low melting point fatty acids (i.e., unsaturated and branched-chain fatty acids) increase membrane fluidity (Welker 1976), whereas high melting point fatty acids (i.e., saturated, long and straight chain fatty acids) decrease membrane fluidity (McElhaney 1976). In general, as growth temperature for bacteria is lowered, membrane lipids contain increasing amounts of unsaturated 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.

The objective of this study was to determine the effect of growth temperatures on the fatty acid profile of *L. monocytogenes*. The purpose of this study was to determine whether the fatty acid profile of *L. monocytogenes* was altered in a systematic way in response to changes in growth temperature.

## MATERIALS AND METHODS

*Listeria monocytogenes* strains Scott A (serotype 4b) and ATCC 19114 (serotype 4a) were used in the study. The strains were obtained from The University of Tennessee Food Technology and Science Department culture collection. Cells were grown on tryptose phosphate agar (TPA; Difco, Detroit, MI) slants at 35C for 24 h and stored at 4C. The cultures were transferred periodically to maintain viability. Tryptose phosphate broth (TPB; Difco) was used to grow cultures prior to all experiments. Peptone (0.1% w/v) was used as a diluent for cultures grown in TPB.

### Analysis of Fatty Acids

Fatty acid composition was determined using a modification of the MIDI Automated Microbial Identification System (MIS) (Sasser 1990). A broth culture (1 ml) was spread on the surface of a TPA plate and incubated in tandem with an inoculated tube of TPB. When the OD<sub>600 nm</sub> of the broth reached 0.6, the plate was removed from incubation for fatty acid analysis. The cell mass from the surface of one plate was removed and transferred to a tube (13 × 100 mm) containing 1.0 ml of Reagent 1 (15% NaOH in 50% methanol). Tubes were sealed with Teflon-lined caps and heated in a boiling water bath for ca. 5 min. Tubes were mixed for 5–10 s and returned to the water bath to complete the 30 min heating. The tube was removed from the bath, cooled to room temperature, and

2 ml of Reagent 2 (325 ml 6.0 N HCl, 275 ml methyl alcohol) was added. The mixture was heated for 10 min at 80C in a water bath, cooled and 1.25 ml of Reagent 3 (hexane, methyl tert-butyl ether; 1:1) added. The tube was gently tumbled on a clinical rotator for about 10 min and the aqueous (lower) phase discarded. About 3 ml of Reagent 4 (0.30 N NaOH) was added to the organic phase and the tube mixed for 5 min. Two-thirds of the organic phase was transferred to a vial, flushed with nitrogen and stored at -18C. Methyl esters were analyzed using a Hewlett Packard (Kennett Square, PA) 5890 Series II Gas Chromatograph on a SPB-1 fused silica capillary column (Supelco, Inc., Bellefonte, PA). Major fatty acids were identified by comparison of retention times with those of pure bacterial fatty acid methyl esters standard (Supelco, Inc.). The chromatographic conditions were as follows: column temperature programmed from 150C to 250C at 4C/min; carrier gas flow linear velocity, 20 cm/s of helium; injection temperature, 250C; detector, flame ionization at 280C; sample size, 5  $\mu$ l of sample extract, 1  $\mu$ l of standard.

### Statistical Analysis

All data were analyzed by analysis of variance using SAS (SAS Institute Inc., Cary, NC). The dependent variables were strains, temperatures, and two replications. Tukey's Multiple-Range Test was used to separate significant differences ( $p < 0.05$ ) among means (Steel and Torrie 1960).

## RESULTS AND DISCUSSION

### Effect of Varying Temperatures on Fatty Acids Composition

Fatty acid compositions of two strains of *Listeria monocytogenes* were surveyed for differences among the type and/or relative percentages of individual fatty acids. This variability could be important since gas-liquid chromatography of fatty acid methyl esters has been used to identify *L. monocytogenes*. For ease of presentation, relative percentages of anteiso-C15:0 (a-C15:0) and iso-C15:0 (i-C15:0) were combined and are indicated as branched-C15:0 (C15:0br). Similarly, iso-C17:0 (i-C17:0) and cyclopropane-C17:0 (C17:0 $\Delta$ ) are reported as branched-C17:0 (C17:0br).

Increasing the temperature from 7C to 35C altered the fatty acid profiles of the two strains of *L. monocytogenes* (Fig. 1 and 2). For strain Scott A, C15:0br decreased with the increase of temperature from 75.7% to 49.8% and for strain ATCC 19114 79.8% to 40.7% at 7 and 35C, respectively. In contrast, C17:0br increased from 9.1% to 33% for strain Scott A (Fig. 1) and 6.3% to 32.7% for

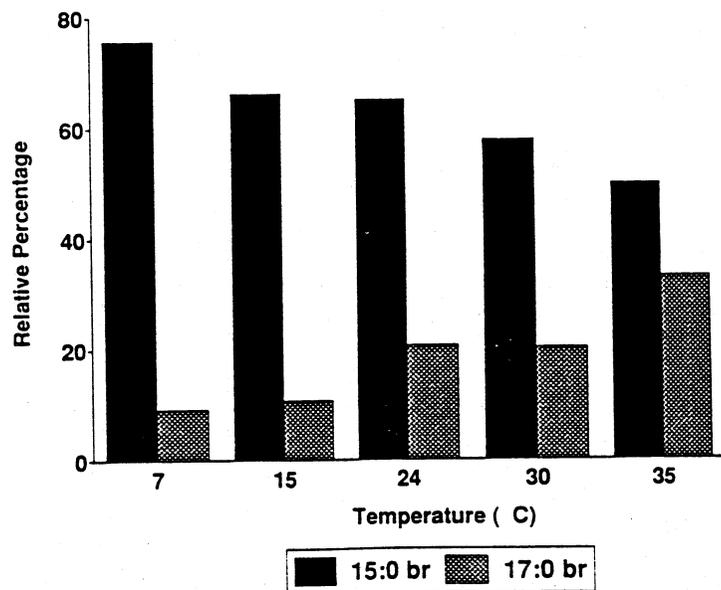


FIG. 1. MEAN RELATIVE PERCENTAGE OF BRANCHED CHAIN FATTY ACIDS FOUND IN *LISTERIA MONOCYTOGENES* STRAIN SCOTT A, GROWN ON TRYPTOSE PHOSPHATE AGAR AT FIVE TEMPERATURES

strain ATCC 19114 (Fig.2). The high relative percentage of C15:0br fatty acid observed at lower growth temperatures likely increased membrane fluidity. However, higher temperature resulted in overall decreased levels of branched chain fatty acids, suggesting decreased membrane fluidity. Thus, cells may resist the effects of high temperature by reducing the relative percentages of branched chain fatty acids.

The overall relative percentages of unknown fatty acid 1, C12:0, C14:0, C15:0, OH-C14:0, and C16:0 decreased with increased temperature (Fig. 3 and 4). For strain Scott A, these fatty acids decreased from 8.6% at 7C to 6% at 35C and, for strain ATCC 19114, from 7.9% to 5.5%, respectively. Fatty acid C12:0 was not detected in strain ATCC 19114 at 15C or 24C (Fig. 4), while this fatty acid was detected in strain Scott A (Fig. 3). In general, most of the medium chain fatty acids decreased as temperature increased.

There was great variation in the types and relative percentages of long chain fatty acids (C18:0 and above) between the two strains at different temperatures (data not shown). For strain Scott A, the total relative percentage of long chain fatty acids was greatest at 15C (4.74%). The percentage decreased at 35C to 2.09%. For strain ATCC 19114, the total relative percentage of long chain fatty acids was greatest at 24C (4.1%) followed by 35C (3.23%). A high percentage

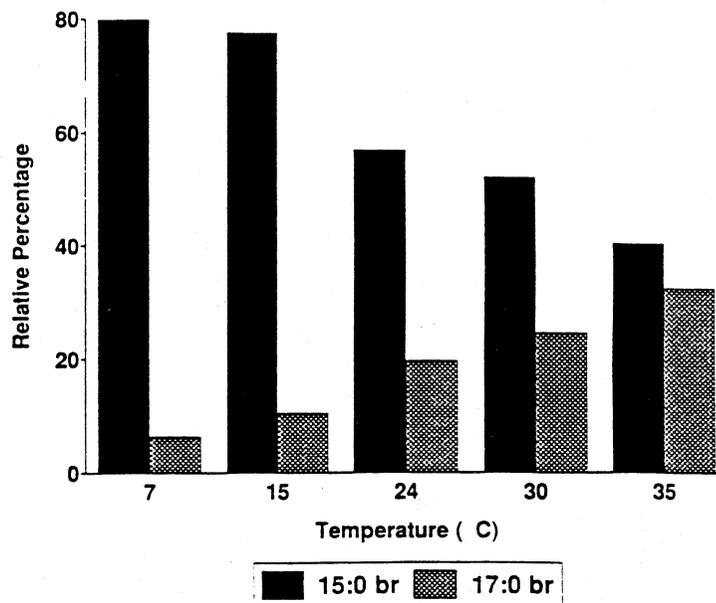


FIG. 2. MEAN RELATIVE PERCENTAGE OF BRANCHED CHAIN FATTY ACIDS FOUND IN *LISTERIA MONOCYTOGENES* STRAIN ATCC 19114, GROWN ON TRYPTOSE PHOSPHATE AGAR AT FIVE TEMPERATURES

of C20:0 was observed at 30C for strain ATCC 19114. Unknown long chain fatty acids were detected in both strains at various temperatures. The relative percentage of these fatty acids was greatest in strain Scott A (7.07%) and ATCC 19114 (13.15%) at 35C.

Major fatty acids identified for the two strains of *L. monocytogenes* agree with results of previous researchers. Raines *et al.* (1968) found C15:0-saturated branched-chain fatty acid as the most abundant fatty acid of *L. monocytogenes*, followed by C14:0, C16:0 and C17:0 branched-chain fatty acids. Other researchers (Ninet *et al.* 1992; Bernard *et al.* 1991; Feresu and Jones 1988; Julak *et al.* 1989; Carrol *et al.* 1968) reported straight- and branched-chain saturated acids, with C15:0 and C17:0 anteiso acids as the most abundant fatty acids in *L. monocytogenes*. Al-issa *et al.* (1984) found that the most abundant acid was anteiso C-15:0 followed by anteiso C-17:0, iso C-15:0 and C-16:0. Tadayon *et al.* (1969) reported that in *L. monocytogenes* the proportion of C17:0br was much lower at 4C than at higher temperatures and the difference was made up mainly by C15:0br. Slight differences observed in these studies could be due to environmental conditions present during growth, i.e., pH, atmosphere, temperature and growth medium.



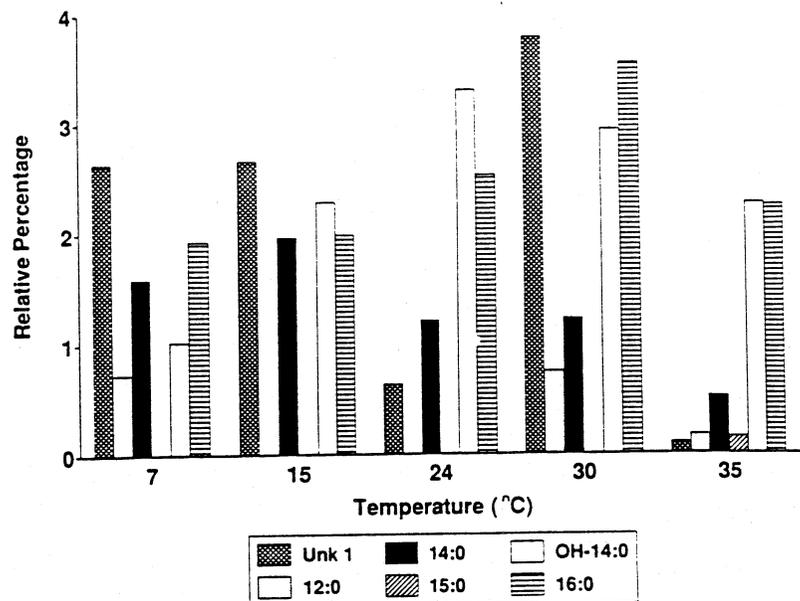


FIG. 4. MEAN RELATIVE PERCENTAGE OF SELECTED MEDIUM CHAIN FATTY ACIDS FOUND IN *LISTERIA MONOCYTOGENES* STRAIN ATCC 19114, GROWN ON TRYPTOSE PHOSPHATE AGAR AT FIVE TEMPERATURES

fatty acids at the expense of the higher-melting branched- and straight-chain fatty acids at lower growth temperatures (Weerkamp and Heinen 1972).

Results of this study clearly demonstrate that the fatty acid profiles of the two strains of *L. monocytogenes* were altered in response to different temperatures. General decreases in relative percentages of branched and medium chain fatty acids and variable changes in long chain fatty acids were found with increasing growth temperature. In addition, cells grown at different temperatures displayed variations in the types and relative percentages of individual fatty acids. Major differences among strains were in relative percentages of saturated branched chain fatty acids (C15:0br and C17:0br) as well as the variable presence of certain long chain fatty acids. Our results indicate that if gas-liquid chromatography is to be used to identify *L. monocytogenes* strains, incubation conditions must be controlled.

#### ACKNOWLEDGMENTS

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