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Use of Fatty Acid Profiles to Identify Food-Borne Bacterial Pathogens and Aerobic Endospore-Forming Bacilli

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Capillary gas chromatography (GC) with flame ionization detection was used to determine the cellular fatty acid profiles of various food-borne microbial pathogens and to compare the fatty acid profiles of spores and vegetative cells of the same endospore-forming bacilli. Fifteen bacteria, representing eight genera (*Staphylococcus*, *Listeria*, *Bacillus*, *Yersinia*, *Salmonella*, *Shigella*, *Escherichia*, and *Vibrio*) and 11 species were used to compare the extracted fatty acid methyl esters (FAMEs). Endospore-forming bacilli were processed to obtain pure spores and whole cell FAMEs for GC analysis. A data set for each bacterial agent was prepared using fatty acid profiles from five replicates prepared on different days. The results showed that these fatty acid intensity profiles were unique for each of the 11 species and that they could be used as a fingerprint for the organisms. The cellular fatty acid profiles for *Bacillus anthracis* and *Bacillus cereus* show that there are two branched chain fatty acids, iso 17:1 ω10c and 17:1 anteiso, which are unique in these species. Iso 17:1 ω10c is present in *B. cereus* vegetative cells and spores but is not observed in *B. anthracis*. The 17:1 anteiso fatty acid is present in *B. anthracis* cells but not in *B. cereus* cells. Fatty acids 16:0 2OH and 17:0 iso 3OH are present in *B. anthracis* and *B. cereus* spores but not in the vegetative cells. In summary, analysis of FAMEs from bacteria and spores can provide a sensitive procedure for the identification of food-borne pathogens.

KEYWORDS: Gas chromatography; fatty acids; food-borne bacteria; spores

INTRODUCTION

In 1963, Able et al. and Kaneda reported that cellular fatty acid methyl esters (FAMEs), analyzed by gas chromatography (GC), could be utilized for the identification of bacteria (*1*, *2*). Subsequent studies also helped to establish the analysis of cellular fatty acids as a method for the identification and classification of bacteria (*3*). Fatty acid analysis has also been used for the taxonomic study of aerobic endospore-forming bacilli (*4*).

Bacillus anthracis and *Bacillus cereus* are closely related pathogenic organisms that are difficult to differentiate genotypically or phenotypically. Vegetative and spore forms of bacilli are morphologically and chemically distinct, but specific chemical markers to distinguish between these species have only recently been described. Fox et al., using GC-MS, showed that vegetative cells and spores of *B. anthracis* and *B. cereus* exhibited distinct carbohydrate profiles (*5*). *B. anthracis* contained high levels of galactose but did not contain galactosamine, whereas *B. cereus* contained galactosamine. Spores exhibited unique carbohydrate profiles as compared with those of vegetative cells. *B. anthracis* spore profiles contained only rhamnose, whereas *B. cereus* spore profiles contained rhamnose and fucose.

Spores and vegetative cells were propagated and harvested from *Bacillus licheniformis*, isolated from raw milk, to obtain fatty acid profiles by gas−liquid chromatography (GLC) (*6*). FAME profiles of 15 bacteria were also investigated using GC and mass spectrometric analysis (*7*). Analytical chemical methods such as vibrational spectroscopy (*8*, *9*) and capillary GC with flame ionization detection (FID) have also been successfully applied to the identification of bacteria and spores (*10*). Recently, infrared spectra using an attenuated total reflection−Fourier transform infrared spectroscopic procedure was used to identify food-borne pathogens (*11*). An automated MIDI commercial system (*12*) is available for identification of microorganisms based on microbial FAME profile databases and pattern recognition software for species determination.

In this study, capillary GC with FID was used to determine the whole cell fatty acid profiles of the majority of the food-borne microbial pathogens compiled by the American Medical Association (AMA), Centers for Disease Control and Prevention (CDC), U.S. Food and Drug Administration (FDA), and Food Safety and Inspection Service of the U.S. Department of Agriculture that are especially important for food-borne illnesses

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Table 1. Bacterial Agents Analyzed by GC

microorganism	designation	source of strains	refs
E. coli[a] O157:H7	933W	clinical hemorrhagic colitis or infantile diarrhea	*25*
E. coli O157:H7	933J	clinical	*25*
E. coli O157:H7	1639-78	clinical hemorrhagic colitis and hemolytic uremic syndrome	*26*
E. coli 153:H2	393	clinical raw milk outbreak, Oregon	W. Hill (FDA)
E. coli O^{-}:H11	402	clinical Washington state outbreak	Phillip Tarr TB334
V. cholerae	2194C	clinical	*27*
V. parahaemolyticus	4037	clinical	*28*
V. vulnificus	C7684	clinical	*29*
L. monocytogenes	ScottA	clinical milk-borne outbreak	*30*
B. anthracis	Sterne	anthrax spore vaccine South Africa	*31*
Y. enterocolitica	289	clinical serotype O:3, biotype 4, phage type VIII	*32*
B. cereus	ATCC 700282	diverse soils	*33*
S. aureus	ATCC 19095	clinical leg abscess, Chicago, IL	*34*
S. typhimurium	DT104	clinical serotype 104B, Hawaii	source H2662
S. sonnei	20143	clinical isolated from human feces in Somalia, 1993	Scott Thorton, U.S. Navy

[a] All *E. coli* strains are enterohemorrhagic.

and to compare the fatty acid profiles of spores and vegetative cells of the same endospore-forming bacilli.

MATERIALS AND METHODS

Bacterial Agents and Growth Conditions. Fourteen bacterial agents were selected from the Foodborne Illnesses Table compiled by the AMA, CDC, FDA, and U.S. Department of Agriculture (*13*). *B. anthracis* Sterne (Colorado Serum Co., Denver, CO) was also included. The 15 bacterial agents are listed in **Table 1**.

All bacteria were grown on brain−heart infusion (BHI) agar (Difco, Detroit, MI) and were initiated from frozen stocks. The growth medium was prepared with 52 g BHI agar/L distilled water, pH 7.0. The formula for BHI agar is as follows: calf brains, infusion from 200 g; beef heart, infusion from 250 g; 10 g of Bacto proteose peptone; 2 g of Bacto dextrose; 5 g of sodium chloride; 2.5 g of disodium phosphate; and 15 g of Bacto agar. For *Vibrio vulnificus*, the medium was also prepared with 1% NaCl. Twenty milliliters of media were added to each 100 mm Petri plate. All bacteria were incubated at 35 ± 1 °C for 24 h.

B. anthracis and *B. cereus* were inoculated into Schaeffer's sporulation medium to induce sporulation (*14*). The culture was vigorously aerated at 35 ± 1 °C for 24 h using a rotary shaker. Incubation was continued for an additional 24 h at 35 ± 1 °C without shaking. The bacterial spores were harvested by centrifugation and washed approximately 20 times in 50 mL of distilled water to remove cellular debris. The purity of the spore preparation was checked by wet mount microscopic examination until no vegetative cells or cellular debris were detected.

Chemical Procedures and GC Analysis. Using a sterile disposable wooden stick, 40−50 mg of bacterial cells was harvested from the culture plates, placed in sterile 13 mm × 100 mm tubes, and weighed on an analytical balance. Approximately 40−50 mg of pure spores was also placed in sterile 13 mm × 100 mm tubes. One milliliter of 3.75 N NaOH (1:1, methanol:distilled water) was added to each tube containing the bacteria or spores to saponify the fatty acids. The tubes were heated in a boiling water bath for 5 min, vortexed, heated for an additional 30 min in a boiling water bath, and then cooled in tap water. Two milliliters of 3.25 N HCl (1:1.18, methanol:6 N HCl) were added for methylation of the fatty acids, and the tubes were heated for 10 min at 80 °C. The tubes were cooled, and the FAMEs were extracted by the addition of 1.25 mL of 1:1 hexane:methyl *tert*-butyl ether with gentle tumbling for 10 min. The lower phase was pipetted off, and 3.0 mL of 0.3 N NaOH was added to the organic phase as a base wash and tumbled for an additional 5 min. The organic phase was then removed for GC analysis.

The FAMES were analyzed by GC using the Microbial Identification System (MIS, MIDI Inc., Newark, DE) software to identify the relative amounts of fatty acids in the bacteria and spore samples and were expressed as a percentage of the total fatty acids. The GC used was a Hewlett-Packard 6890 with a FID and a HP7683 autosampler (Hewlett-Packard Co., Palo Alto, CA). A 25 m × 0.2 mm cross-linked 5% phenylmethyl silicone fused silica capillary column (HP 19091B-102) was used to separate the fatty acids. Operating conditions were as follows: The initial temperature was 170 °C and was increased at a rate of 5 °C/min to 260 °C and then increased to 310 °C at 40 °C/min and held for 1 min. Hydrogen was used as the carrier gas at a flow rate of 0.4 mL/min. A calibration analysis was used for the first two injections of every sequence and was automatically reanalyzed after every 11th sample injection using Calibration Standard 1 (MIDI, Inc.). FAME standards and GC-MS were used in identifying peaks.

Electron Microscopy. Samples of *B. anthracis* and *B. cereus*, grown for 30 h at 37 °C on BHI agar containing 50 mg Mn/L, were immersed for 15 h at 4 °C in a fixative containing 4% paraformaldehyde, 1% glutaraldehyde, and 0.1 M sodium cacodylate (pH 7.2). The samples were rinsed three times in cold 0.1 M cacodylate buffer and postfixed for 15 h in a cold 1% solution of osmium tetroxide in 0.1 M sodium cacodylate buffer. The specimens were dehydrated, embedded with Spurr low-viscosity epoxy medium, and polymerized by heating at 60 °C for 24 h. Ultrathin sections of the specimens were prepared and doubly stained using 2% uranyl acetate and lead citrate (*15*). The sections were examined using a Zeiss EM10 electron microscope.

Statistical Analysis. Differences in fatty acids among bacteria were assessed by analysis of variance (ANOVA) (*16*). The Duncan multiple comparison method was used to differentiate among means for fatty acids that were significantly different for various bacteria (*16*). Values are expressed as means with their standard errors.

Data analysis and determination of clustering were performed by principal component analysis (PCA) using PIROUETTE 3.0 software (InfoMetrix, Bothell,WA). Data were compiled as Excel spreadsheets and imported into PIROUETTE as a data matrix for which each bacterial sample consisted of a row and each variable (fatty acid relative concentration) consisted of a column. Each sample represents a single point in the multivariate space defined by the set of independent axes corresponding to each fatty acid concentration.

The principal components (PCs) are a new set of orthogonal axes that are rotated from the original variable axes. The PCs are weighted linear combinations of the original variables in the data and are computed such that the first PC accounts for the maximum variation (variance) in the data. Each subsequent computed PC accounts for the maximum variation remaining in the data, and the PCs are orthogonal to one another. This leads to a reduction in the dimensionality of the data by using the most significant PCs to represent the data in fewer dimensions. Clustering in the data is observed through the use of scores plots in which the data for each sample are projected onto the reduced set of PCs. For PCA, the data were scaled by mean centering; PCs were computed using the Nipals Method (Pirouette Manual), and the first three PCs accounted for approximately 94% of the total variance in the data. The two-dimensional scores plots are scatter plots depicting PC1 vs PC2 using range-dependent distance scaling.

RESULTS AND DISCUSSION

GLC-FID analysis of chemical components of bacterial cells has provided useful information for rapid detection and iden-

Table 2. Comparison of Cellular Fatty Acid Profiles for *B. anthracis* and *B. cereus*[a]

	B. anthracis (%)		*B. cereus* (%)		
cellular fatty acid	vegetative cells	spores	vegetative cells	spores	*P* value
12:0 Iso			0.68 ± 0.18^{b}	0.29 ± 0.19^{a}	0.005
13:0 Iso	2.87 ± 0.12^{a}	5.02 ± 0.07^{b}	5.54 ± 0.11^{b}	5.91 ± 0.90^{b}	0.001
13:0 Anteiso	0.44 ± 0.04^{a}	0.72 ± 0.20^{b}	0.13 ± 0.13^{a}	1.10 ± 0.26^{b}	0.018
14:0 Iso	1.52 ± 0.39^{a}	3.42 ± 0.12^{b}	5.12 ± 0.27^{c}	4.11 ± 0.22^{b}	<0.001
14:0	2.29 ± 0.11^{b}	2.03 ± 0.05^{a}	3.40 ± 0.05^{c}	3.46 ± 0.07^{c}	<0.001
15:0 Iso	42.00 ± 1.30^{d}	26.16 ± 0.38^{b}	133.27 ± 1.15^{c}	9.27 ± 1.39^{a}	<0.001
15:0 Anteiso	4.93 ± 0.18^{b}	8.39 ± 0.13^{d}	2.74 ± 0.09^{a}	6.97 ± 0.44^{c}	<0.001
16:0 Iso	6.95 ± 0.30^{a}	11.06 ± 0.23^{b}	12.62 ± 0.48^{c}	7.29 ± 0.56^{a}	<0.001
16:0	5.73 ± 0.34^{a}	6.11 ± 0.22^{a}	7.37 ± 0.11^{b}	7.57 ± 0.53^{b}	0.003
Iso 17:1 ω10c			0.87 ± 0.36^{b}	1.39 ± 0.61^{b}	0.032
Iso 17:1 ω5c	3.05 ± 0.10^{a}	2.04 ± 0.54^{a}	3.04 ± 0.12^{a}	5.36 ± 0.81^{b}	0.002
17:1 Anteiso	1.42 ± 0.06^{b}	2.96 ± 0.28^{c}		0.55 ± 0.34^{a}	<0.001
17:0 Iso	12.04 ± 0.49^{c}	10.84 ± 0.23^{b}	11.99 ± 0.37^{c}	6.16 ± 0.23^{a}	<0.001
17:0 Anteiso	3.74 ± 0.11^{b}	8.77 ± 0.11^{c}	0.92 ± 0.38^{a}	3.03 ± 0.41^{b}	<0.001
16:0 2OH		2.47 ± 0.09^{b}		2.71 ± 0.27^{b}	<0.001
18:1 ω5c		0.40 ± 0.40		0.83 ± 0.52	0.265
18:0	0.47 ± 0.35				0.202
17:0 Iso 3OH		3.08 ± 0.19^{b}		3.20 ± 0.27^{b}	<0.001
summed 16:1 Iso I/14:0 3OH	1.85 ± 0.06^{a}	1.20 ± 0.49^{a}	3.39 ± 0.12^{b}	1.79 ± 0.05^{a}	<0.001
summed 15:0 Iso 2OH/16:1 ω7c	7.86 ± 0.24^{b}	5.33 ± 0.30^{a}	8.60 ± 0.10^{b}	18.32 ± 1.10^{c}	<0.001
summed 18:2 ω6, 9c/18:0 Anteiso	0.86 ± 0.37				0.009

[a] Values are the means ± SEM; $n = 5$. Means for a variable not sharing a common letter are significantly different ($P < 0.05$), as determined by the Duncan multiple comparison method, which was applied only if significant differences were determined to exist by ANOVA.

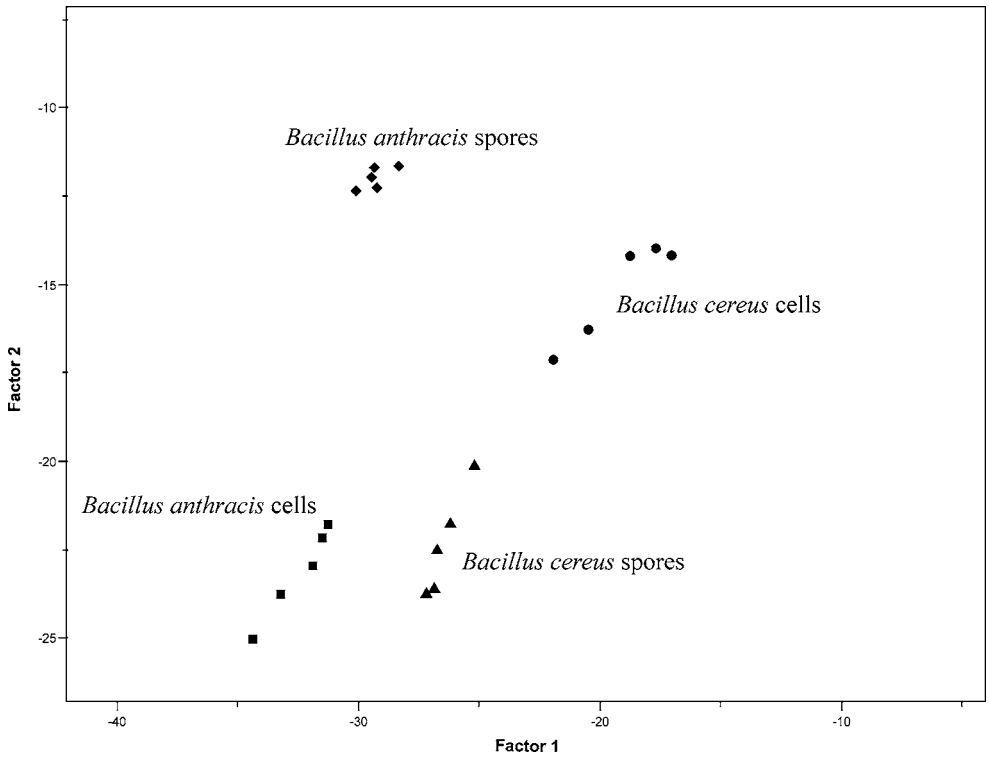

Figure 1. Cellular fatty acid clustering of *B. anthracis* vegetative cells, *B. cereus* vegetative cells, *B. anthracis* spores, and *B. cereus* spores as determined by PCA with full data set, mean center scaling, and expanded view.

tification of bacteria in clinical and diagnostic bacteriology laboratories and currently has increased significance for both food safety and security. In this study, the bacteria listed in **Table 1** were grown on BHI agar, a highly nutritive medium used for cultivating a variety of fastidious microorganisms. Spores were induced in *B. anthracis* and *B. cereus* by inoculation into Schaeffer's sporulation media. We extracted the fatty acids from the whole bacterial cells and spores, derivatized them into methyl esters, and then identified and quantified the FAMEs using GC-FID.

The cellular fatty acid profiles for *B. anthracis* and *B. cereus* show that among the group of bacteria that we analyzed, there are two fatty acid profiles consistent with iso 17:1 ω10c and 17:1 anteiso, which are unique for these species. Iso 17:1 ω10c is present in *B. cereus* vegetative cells and spores but is not observed in either *B. anthracis* cells or *B. anthracis* spores. In contrast, 17:1 anteiso fatty acid is present in *B. anthracis* cells but not in *B. cereus* cells. Additionally, fatty acid profiles consistent with 16:0 2OH and 17:0 iso 3OH are present in *B. anthracis* and *B. cereus* spores but not in the vegetative cells

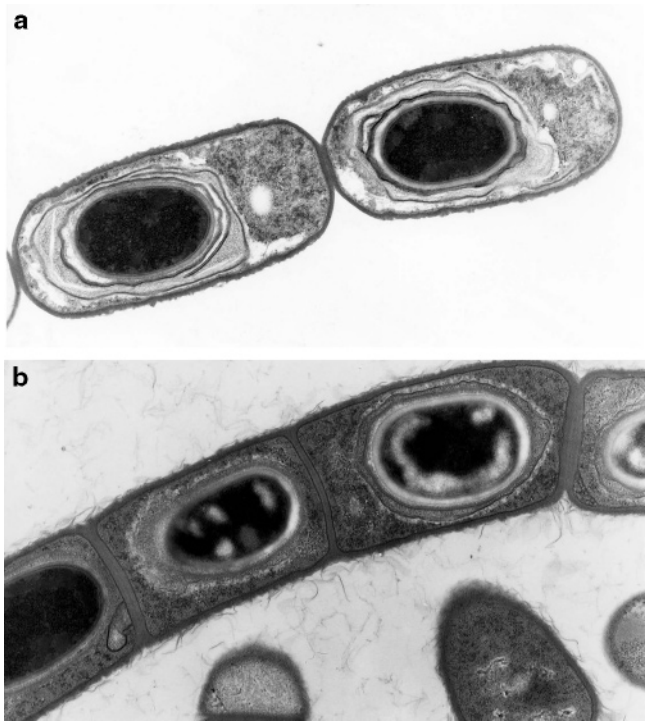

Figure 2. (**a**) Longitudinally sectioned *B. cereus* cells growing in long chains and showing developing spores (×40000). (**b**) Longitudinally sectioned *B. anthracis* cells growing in long chains and showing developing spores (×40000).

(**Table 2**). As shown in **Figure 1**, PCA of the cellular fatty acids of *B. anthracis* vegetative cells, *B. cereus* vegetative cells, *B. anthracis* spores, and *B. cereus* spores predicts the clustering of the vegetative cells and spores. These analyses are important in showing that *B. anthracis* and *B. cereus* cells can easily be identified and distinguished from one another when grown on BHI agar. In addition, *B. anthracis* spores and *B. cereus* spores can be identified and differentiated based on fatty acid data. This may be especially significant because *B. anthracis* can be directly identified without the need for spore germination.

The spores of *B. anthracis* and *B. cereus* also have higher percentages of anteiso fatty acid profiles consistent with 13:0, 15:0, 17:1, and 17:0 when compared to *B. anthracis* and *B. cereus* cells. The *B. anthracis* spores have significantly higher percentages of anteiso fatty acid profiles consistent with 15:0, 17:1, and 17:0 when compared to *B. cereus* spores. When comparing the total iso fatty acids, the vegetative cells of *B. anthracis* and *B. cereus* have a slightly higher percentage than the corresponding spores. The two iso fatty acids contributing the highest percentages were 15:0 iso and 17:0 iso, which were both significantly higher for *B. anthracis* and *B. cereus* vegetative cells. Song et al. (*10*) also examined spore and vegetative cellular fatty acid profiles of bacilli. However, a different medium was used for growing the vegetative cells and a different procedure was followed for spore formation resulting in the production of a differing fatty acid profile.

Both *B. anthracis* and *B. cereus* are Gram-positive spore-forming rods and tend to grow in long chains. **Figure 2a**,**b** shows electron micrographs of the strains used in this study and shows differences in the junctions between the cells and the hairlike projections on the cell surface of *B. anthracis*. The spores are characterized by nonswelling of the sporangium. *B. anthracis*, the etiological agent of anthrax, has spores that are highly resistant to adverse environmental conditions including heat, pressure, and ultraviolet and ionizing radiation (*17*). *B. cereus*, the organism most easily mistaken for *B. anthracis*, has been isolated from a wide variety of raw and processed foods but is not considered a significant health threat unless it is able to proliferate and produce toxins. The consumption of foods contaminated with greater than 10^5 viable enterotoxigenic *B. cereus* cells per gram of food has resulted in outbreaks of food poisoning (*18*).

Table 3 shows the FAME profiles for *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *V. vulnificus* without and with 1% NaCl added to the media. Because *V. vulnificus* is a marine organism, 1% NaCl was added to improve growth and to allow a comparison of results obtained without the added NaCl. *V. cholerae* had a significantly higher amount of fatty acid 14:0 than the other *Vibrios*, and 14:0 iso, 14:0 iso 3OH, and 11

Table 3. Comparison of Cellular Fatty Acid Profiles for Various Strains of *Vibrio* Species[a]

cellular fatty acid	*V. cholerae*	*V. parahaemolyticus*	*V. vulnificus*	*V. vulnificus* (1% NaCl)	*P* value
10:0 3OH	0.22 ± 0.01^{b}		0.11 ± 0.03^{a}	0.24 ± 0.01^{b}	<0.001
12:0		3.25 ± 0.10			<0.010
unknown 12.484	0.95 ± 0.04	0.88 ± 0.04	0.88 ± 0.12	0.87 ± 0.03	0.813
12:0 2OH	0.16 ± 0.01		0.12 ± 0.04	0.14 ± 0.01	0.306
12:1 3OH	0.13 ± 0.01^{a}		5.48 ± 0.50^{b}	0.12 ± 0.03^{a}	<0.001
12:0 3OH	6.66 ± 0.25^{c}	2.21 ± 0.08^{a}		5.32 ± 0.16^{b}	<0.001
14:0 Iso	0.11 ± 0.05				0.012
14:0	8.99 ± 0.21^{d}	6.28 ± 0.09^{c}	5.80 ± 0.13^{b}	5.19 ± 0.05^{a}	<0.001
14:0 Iso 3OH	0.23 ± 0.12				0.030
16:1 ω7c OH	0.64 ± 0.04^{c}		0.08 ± 0.05^{a}	0.31 ± 0.01^{b}	<0.001
16:0 Iso	0.88 ± 0.27^{b}	0.05 ± 0.05^{a}	0.16 ± 0.08^{a}		0.002
16:1 ω9c	0.85 ± 0.01^{b}		0.48 ± 0.12^{a}		<0.001
16:1 ω5c		0.11 ± 0.07	0.20 ± 0.08	0.20 ± 0.08	0.262
16:0	26.71 ± 0.25^{a}	28.23 ± 0.32^{b}	29.47 ± 0.57^{c}	$28.66 \pm 0.20^{b,c}$	0.001
17:1 ω8c	0.23 ± 0.02^{a}	0.61 ± 0.07^{b}	0.13 ± 0.05^{a}		<0.001
17:0	0.24 ± 0.02^{a}	1.06 ± 0.14^{b}	0.40 ± 0.06^{a}		<0.001
18:1 ω9c	0.41 ± 0.02^{b}		0.03 ± 0.03^{a}		<0.001
18:1 ω7c	13.79 ± 0.14^{a}	13.86 ± 0.07^{a}	17.86 ± 0.34^{c}	15.89 ± 0.18^{b}	<0.001
18:0	1.19 ± 0.01^{b}	1.02 ± 0.03^{a}	1.14 ± 0.04^{b}	1.12 ± 0.02^{b}	0.004
11 methyl 18:1 ω7c	0.35 ± 0.02				<0.001
summed 14:0 3OH/16:1 Iso	6.10 ± 0.24^{b}	4.21 ± 0.15^{a}	7.68 ± 0.70^{c}	7.31 ± 0.18^{c}	<0.001
summed 15:0 Iso 2OH/16:1 ω7c	30.59 ± 0.25^{a}	34.73 ± 0.50^{b}	29.19 ± 0.96^{a}	33.97 ± 0.13^{b}	<0.001

[a] Values are the means ± SEM; $n = 5$. Means for a variable not sharing a common letter are significantly different ($P < 0.05$) as determined by the Duncan multiple comparison method, which was applied only if significant differences were determined to exist by ANOVA.

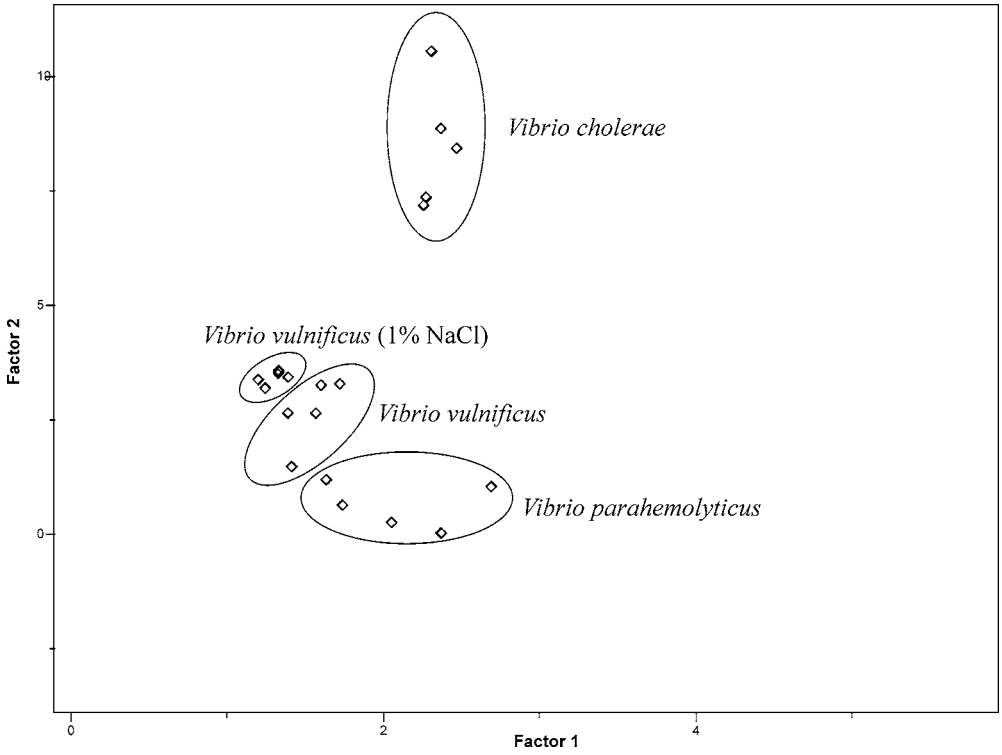

Figure 3. Cellular fatty acid clustering of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* as determined by PCA, full data set, autoscaling, and expanded view.

Table 4. Comparison of Cellular Fatty Acid Profiles for Various Enterohemorrhagic Strains of *E. coli*[a]

	E. coli					
cellular fatty acid	O157:H7 933W	O157:H7 933J	O157:H7 1639–78	153:H2 393	O$^-$:H11 402	*P* value
12:0	4.09 ± 0.10^b	4.09 ± 0.13^b	3.77 ± 0.06^a	4.07 ± 0.08^b	3.73 ± 0.03^a	0.010
13:0	0.06 ± 0.04^a	0.18 ± 0.01^{a,b}	0.65 ± 0.07^c	0.21 ± 0.05^b	0.91 ± 0.03^d	<0.001
14:0	5.50 ± 0.20^a	5.54 ± 0.08^a	7.92 ± 0.06^c	8.36 ± 0.11^d	5.93 ± 0.04^b	<0.001
unknown 14.502	0.81 ± 0.02	0.89 ± 0.03	0.83 ± 0.08	0.84 ± 0.08	0.69 ± 0.02	0.143
15:1 ω8c			0.09 ± 0.04^a		0.23 ± 0.01^b	<0.001
16:1 ω5c				0.02 ± 0.02^a	0.15 ± 0.04^b	<0.001
16:0	37.83 ± 0.41^d	35.84 ± 0.53^c	30.96 ± 0.70^b	34.80 ± 0.67^c	28.00 ± 0.10^a	<0.001
15:0 Iso 3 OH			0.05 ± 0.05^a		0.27 ± 0.01^b	<0.001
17:1 ω8c			0.08 ± 0.08^a		0.60 ± 0.04^b	<0.001
17:0 ω Cyclo 7–8	15.22 ± 0.30^c	10.04 ± 0.73^a	13.54 ± 0.50^{b,c}	15.12 ± 1.19^c	11.83 ± 0.32^{a,b}	<0.001
17:0	1.36 ± 0.06^b	1.87 ± 0.08^c	2.06 ± 0.13^c	0.72 ± 0.10^a	2.83 ± 0.06^d	<0.001
18:1 ω7c	7.63 ± 0.74^a	18.90 ± 1.59^c	14.71 ± 0.39^b	12.08 ± 0.86^b	18.00 ± 0.29^c	<0.001
18:0	1.14 ± 0.03^b	1.31 ± 0.05^c	0.38 ± 0.02^a	0.42 ± 0.02^a	0.45 ± 0.09^a	<0.001
19:0 Cyclo ω8c	13.94 ± 0.67^c	5.44 ± 1.16^b	2.54 ± 0.41^a	4.92 ± 0.59^b	1.58 ± 0.09^a	<0.001
summed 13:0 3OH/15:1 Iso			0.56 ± 0.14^a		0.98 ± 0.05^b	<0.001
summed 14:0 3OH/16:1 Iso	9.62 ± 0.26	9.69 ± 0.35	9.07 ± 0.13	9.14 ± 0.17	8.83 ± 0.19	0.068
summed 15:0 Iso 2OH/16:1 ω7c	2.46 ± 0.30^a	6.17 ± 0.50^b	12.67 ± 0.74^d	9.03 ± 1.39^c	14.93 ± 0.44^d	<0.001

[a] Values are the means ± SEM; $n = 5$. Means for a variable not sharing a common letter are significantly different ($P < 0.05$), as determined by the Duncan multiple comparison method, which was applied only if significant differences were determined to exist by ANOVA.

methyl 18:1 ω7c were observed only in *V. cholerae*. *V. parahaemolyticus* differed from the other *Vibrio* species because of a significantly higher percentage of 17:0, and it was the only *Vibrio* with the fatty acid 12:0. *V. vulnificus* had a significantly higher amount of 12:1 3OH and 18:1 ω7c. With the addition of 1% NaCl, fewer fatty acids were observed. The major change was with the fatty acids 12:1 3OH and 12:0 3OH; the percentages were reversed when cells were grown without the addition of 1% NaCl as compared to the cells grown in the presence of 1% NaCl (**Table 3**). **Figure 3** shows the cellular fatty acid clustering of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* as determined by PCA.

Vibrios are present in the flora of aquatic environments, and most human infections are acquired by exposure to water or consumption of foods harvested from such environments. *V. vulnificus* is responsible for approximately 95% of all seafood-related deaths in the United States (*19*). Risk factors predisposing one to infection with *V. vulnificus* include the consumption of raw seafood by individuals who are immunocompromised or have liver disease and/or iron overload. Impaired iron metabolism and increased iron availability appear to underlie the pathogenicity of *V. vulnificus* (*20*).

The cellular fatty acid profiles for the five strains of *Escherichia coli* are shown in **Table 4**. The types of fatty acids

Table 5. Comparison of Cellular Fatty Acid Profiles for Various Strains of Gram-Negative and Gram-Positive Bacteria[a]

	Gram-negative			Gram-positive	
cellular fatty acid	*Y. enterocolitica*	*S. typhimurium*	*S. sonnei*	*S. aureus*	*L. monocytogenes*
12:0	4.20 ± 0.37	3.49 ± 0.17	3.79 ± 0.14		
13:0 Iso				0.21 ± 0.01	
13:0 Anteiso					0.18 ± 0.05
13:0		0.24 ± 0.02	1.34 ± 0.13		
unknown 13.957		0.77 ± 0.19			
14:0 Iso				1.37 ± 0.02	0.74 ± 0.02
14:0	5.87 ± 0.39	5.29 ± 0.35	7.83 ± 0.10	0.38 ± 0.01	0.72 ± 0.02
unknown 14.502	0.77 ± 0.08	0.96 ± 0.05	0.70 ± 0.04		
14:0 2OH		1.49 ± 0.04			
15:0 Iso				11.78 ± 0.27	13.91 ± 0.09
15:0 Anteiso				42.69 ± 0.27	48.24 ± 0.38
15:1 ω6c			0.40 ± 0.06		
16:0 Iso				1.85 ± 0.03	2.48 ± 0.04
unknown 15.669					0.81 ± 0.68
16:0	41.82 ± 4.27	33.65 ± 0.17	29.44 ± 0.34	2.42 ± 0.05	1.90 ± 0.04
15:0 Iso 3 OH			0.37 ± 0.03		
17:1 ω8c			0.59 ± 0.06		
17:1 ω Cyclo 7–8	25.27 ± 2.44	14.06 ± 0.29	10.31 ± 0.46		
17:0 Iso				7.48 ± 0.12	3.46 ± 0.06
17:0 Anteiso				16.59 ± 0.19	27.25 ± 0.29
17:0	1.54 ± 0.07	1.29 ± 0.02	2.14 ± 0.17	0.46 ± 0.02	
18:1 ω7c	2.63 ± 0.22	15.40 ± 0.20	14.41 ± 0.15		
18:0 Iso				0.92 ± 0.05	
18:0	0.94 ± 0.06	0.42 ± 0.01	0.31 ± 0.01	6.73 ± 0.11	
19:0 Iso				1.96 ± 0.13	
19:0 Anteiso				2.29 ± 0.19	
19:0				0.59 ± 0.03	
19:0 Cyclo ω8c	0.14 ± 0.08	3.78 ± 0.20	0.64 ± 0.17		
20:0				2.18 ± 0.07	
summed 13:0 3OH/15:1 Iso			1.20 ± 0.10		
summed 14:0 3OH/16:1 Iso	9.37 ± 0.32	8.59 ± 0.19	8.78 ± 0.37		
summed 15:0 Iso 2OH/16:1 ω7c	7.20 ± 0.81	10.42 ± 0.26	17.47 ± 0.96		
summed 18:0 Anteiso/18:2 ω6,9c					0.30 ± 0.13

[a] Values are the means ± SEM; $n = 5$.

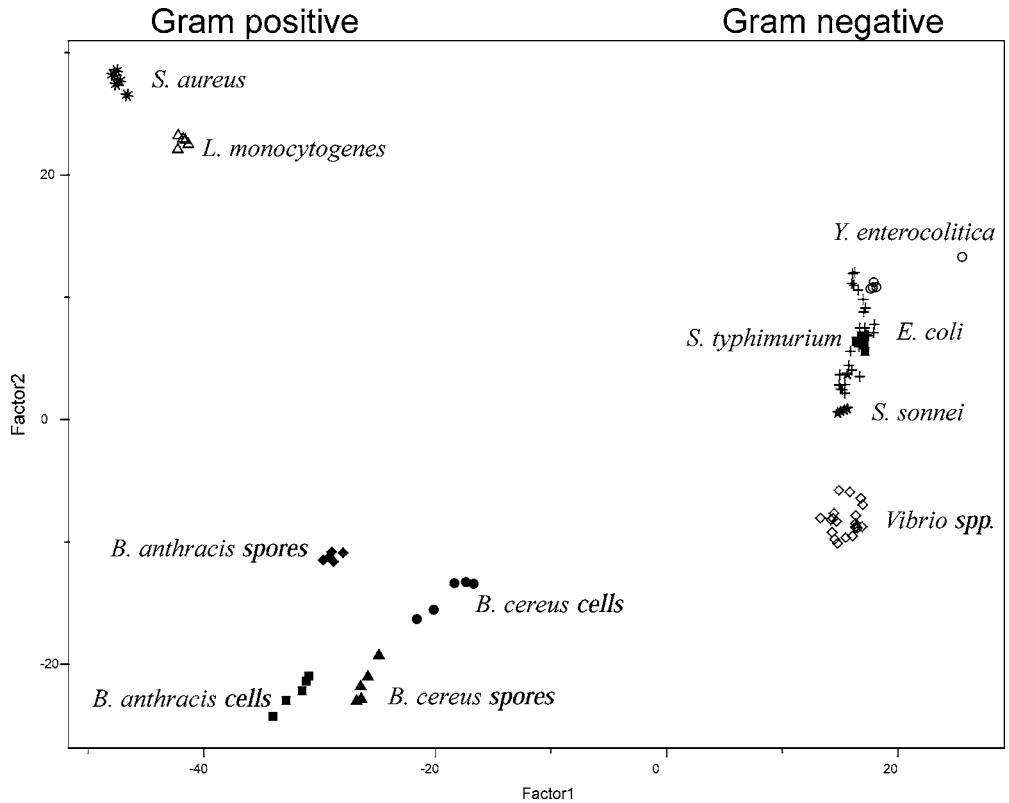

Figure 4. Cellular fatty acid clustering of Gram-positive bacteria *B. anthracis* cells and spores, *B. cereus* cells and spores, *L. monocytogenes*, and *S. aureus* are differentiated from Gram-negative bacteria *Y. enterocolitica*, *E. coli*, *S. typhimurium*, *S. sonnei*, and *Vibrio* spp. as determined by PCA with full data set and mean center scaling.

are similar among the strains, but with some of the fatty acids, there are differences in the percentages. *E. coli* O^{-}:H11 contained the largest number of fatty acids, small percentages of 15:1 ω8c, 16:1 ω5c, and 17:1 ω8c, and slightly higher levels

of 17:0. *E. coli* 0157:H7 (933W) had a significantly higher amount of 19:0 cyclo ω8c and 16:0 than the other strains but was significantly lower in 18:1 ω7c. Enterohemorrhagic *E. coli* (EHEC) may cause severe bloody diarrhea (hemorrhagic colitis) and hemolytic uremic syndrome (*21*, *22*). In the United States, hemolytic uremic syndrome is the major cause of acute kidney failure in young children and the elderly (*21*).

A comparison of cellular fatty acid profiles for three Gram-negative (*Yersinia enterocolitica*, *Salmonella typhimurium*, and *Shigella sonnei*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) is shown in **Table 5**. The Gram- negative bacteria contained straight chain saturated fatty acid profiles (12:0, 14:0, 16:0, and 17:0) and cyclopropane fatty acid profiles (17:1 ωcyclo 7-8 and 19:0 cyclo ω8c). In contrast, the Gram-positive bacteria contained branched iso fatty acid profiles (14:0 iso, 15:0 iso, 16:0 iso, 17:0 iso, 18:0 iso, and 19:0 iso) where the methyl group is on the second to the last carbon in the chain, and branched anteiso fatty acids (15:0 anteiso, 17:0 anteiso, and 19:0 anteiso) where the methyl group is on the third to the last carbon in the chain.

Y. enterocolitica is a food-borne pathogen that enters the body through the gastrointestinal tract. *Y. enterocolitica* withstands freezing and can survive in frozen foods for extended periods (*23*). *L. monocytogenes* causes a food-borne disease that has a fatality rate as high as 30−40%. A variety of foods, such as coleslaw, milk, cheese, hot dogs, and deli turkey meat, have been implicated in major listeriosis outbreaks (*24*). *S. aureus* in foods presents a potential hazard because many strains produce heat resistant enterotoxins. Foods commonly associated with *S. aureus* food poisoning include meat, salads, and dairy products.

The cellular fatty acid clustering of Gram-positive bacteria *B. anthracis* and spores, *B. cereus* and spores, *L. monocytogenes*, *S. aureus*, and the Gram-negative bacteria *Y. enterocolitica*, *E. coli*, *S. typhimurium*, *S. sonnei*, and *Vibrio* spp. as determined by PCA is shown in **Figure 4**. There is a clear separation between the Gram-positive and the Gram-negative bacteria based on their fatty acid profiles.

In summary, this study demonstrates the importance of being able to construct a unique library for the identification of various types of bacteria and spores based on the type of media and specific growing conditions. The unique fatty acids isolated from bacteria and spores can provide a sensitive procedure for the identification of food-borne pathogens. This analysis of FAMEs can be an additional confirmatory technique for biological pathogens together with PCR methods, γ-phage inactivation, and direct fluorescent antibody test.

Safety. Standard microbiological safety precautions were observed while working with the bacteria strains. While performing experimental procedures, safety glasses, laboratory coats, and disposable gloves were utilized. The bacteria were completely inactivated using sodium hydroxide that was heated in a boiling water bath before the fatty acid extraction procedure.

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