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Evaluation of multiple strains of *Enterobacter sakazakii* using fatty acid profiles

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Abstract

Fatty acid profiles are useful for identifying Gram-negative *Enterobacter sakazakii* strains within the family *Enterobacteriaceae*. The majority of cases of *E. sakazakii* infection have involved sepsis, meningitis, or enteritis, especially in neonates and infants. Gas chromatography with flame ionization detection (GC-FID) was utilized for the analysis of cellular fatty acid methyl esters (FAMEs). Thirty *E. sakazakii* strains isolated from food and environmental sources were cultured for 24 h on brain heart infusion (BHI) agar on three different days at 37 °C. Whole cell FAMEs were obtained by saponification, methylation and extraction into hexane:methyl *tert*-butyl ether. The day to day variations for the 30 *E. sakazakii* strains for each fatty acid were determined. Gram-negative bacteria commonly contain combinations of straight-chain, unsaturated, hydroxyl, and *cyclo* fatty acids. Major fatty acids of *E. sakazakii* strains evaluated in this study were straight chain 12:0, 14:0, 16:0 and unsaturated 16:1, 18:1, and 17:0 ω *cyclo* 7–8. Analysis of FAMEs from *E. sakazakii* strains grown on BHI agar by this rapid GC-FID method is highly reproducible and provides a sensitive procedure for identification of this organism. The fatty acid profile assay could be used to rapidly screen infant formula samples for *E. sakazakii* and reduce the time required for the current assay by up to 5 days.
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Keywords: Gas chromatography; Fatty acids; *Enterobacter sakazakii*

1. Introduction

Enterobacter sakazakii is a Gram-negative rod-shaped bacterium of the family *Enterobacteriaceae*. The foodborne pathogen was designated as a yellow-pigmented variant of *Enterobacter cloacae* until 1980 when it was renamed *E. sakazakii* (Farmer, Hickman, & Brenner, 1980). This reclassification was based on differences in DNA–DNA hybridization, pigment production, biochemical reactions, and antibiotic susceptibility when compared to *E. cloacaei* (Farmer et al., 1980). Urmenyi and Franklin (1961) reported the first known cases of meningitis caused by *E. sakazakii* in 1958, when an outbreak in England resulted in the deaths of two infants. The presence of *E. sakazakii* in powdered infant milk formula has been associated with outbreaks of infant meningitis, necrotizing enterocolitis, bacteremia and neonatal deaths (Himelright, Harris, Lorch, & Anderson, 2002; Lai, 2001; van Acker et al., 2001). Since 1961, approximately 70 cases of neonatal infections have been reported worldwide (Drudy, Mullane, Quinn, Wall, & Fanning, 2006). *E. sakazakii* has been isolated from the environment and food, especially in milk powders, cheese products and baby foods (Iversen and Forsythe, 2004). *E. sakazakii* has also been cultured from minced beef, sausage meat and vegetables (Leclercq, Wanegue, & Baylac, 2002).

The current US Food and Drug Administration (FDA) method for the isolation and identification of *E. sakazakii*

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requires two enrichment steps, the subculturing of the second enrichment broth on a selective agar, a further subculturing of selected grown colonies and biochemical identification of yellow-pigmented colonies (Lehner et al., 2006). The FDA method requires 5 days or longer and is not selective for *E. sakazakii* (Drudy et al., 2006). However, the FDA is in the process of developing a rapid real-time PCR method for the specific detection of *E. sakazakii* in powdered milk-based infant formula. Recently, Whittaker, Keys, Brown, and Fry (2007) showed that *E. sakazakii* can be identified by analysis of cellular fatty acid methyl esters (FAMEs) by gas chromatography with flame ionization detection (GC-FID). This method provides a confirmatory procedure in addition to a rapid real-time PCR method (Seo & Brackett, 2005). The percentages and unique fatty acids isolated from *E. sakazakii* can provide a rapid and sensitive method for identification of this bacterium. In this study, the rapid GC-FID method was used to determine if it could reproducibly identify 30 different *E. sakazkii* strains cultured from food and the environment on three different days, and to calculate the day to day variations for each fatty acid percentage and predictability for the *E. sakazakii* strains.

2. Materials and methods

2.1. Bacterial agents and growth conditions

Thirty *E. sakazakii* strains from an FDA collection were analyzed (Table 1). The bacteria were cultured on brain–heart infusion (BHI) agar (Difco, Detroit, MI, USA). The growth medium was prepared with 52 g of BHI agar/L distilled water, pH 7.0. Twenty milliliters of medium was added to each 100 mm Petri plate. Cultures were initiated on three different days and were incubated at 37 ± 1 °C for 24 h.

2.2. Chemical procedures and gas chromatography analysis

For gas chromatography with flame ionization detection (GC-FID) analysis, bacterial cells were harvested from the culture plates and whole cell fatty acid methyl esters (FAMEs) were obtained by saponification, methylation and extraction into hexane:methyl *tert*-butyl ether. Using a sterile disposable wooden stick, approximately 25 mg of bacterial cells were harvested from the culture plates and placed in sterile 13 × 100 mm tubes. One milliliter of 3.75 mol/L NaOH (1:1, methanol/distilled water) was added to each tube containing the bacteria 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 mol/L HCl (1:1.18, methanol/6 mol/L HCl) was 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 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 mol/L 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 rapid Microbial Identification System (MIS, MIDI Inc., Newark, DE, USA) software (RCLN50) to identify the relative amounts of fatty acids in the bacteria, and were expressed as a percentage of the total fatty acids. The GC used was an Agilent 6890 with an FID and an Agilent auto sampler and injector (Agilent 7683) (Agilent Technologies, Palto Alto, CA, USA). A 25 m (length) × 0.2 mm ID × 0.33 μm film thickness, cross-linked 5% phenylmethyl silicone fused silica capillary column (Agilent 19091B-102) was used to separate the fatty acids. Operating conditions were as follows: initial temperature was 170 °C and was increased at a rate of 28 °C/min to 288 °C, and then increased to 310 °C at 60 °C/min and held for 1.25 min. Hydrogen was used as the carrier gas at a constant flow rate of 1.3 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 (No. 1300-AA; Microbial ID, Inc., Newark, DE, USA). The similarity index (SI) is a numerical value that expresses how closely the fatty acid composition of an unknown compares with the mean fatty acid composition of the strains used to create the library entry. As each fatty acid varies from the mean percentage, the SI

Table 1
Bacterial agents analyzed by GC

Microorganism	Designation	Source of strains
Enterobacter sakazakii	2313	Environmental – dry blend facility
	2314	Food – organic soy isolate
	2315	Food – organic soy isolate
	2316	Food – organic soy isolate
	2317	Food – soy flour ingredient
	2318	Food – soy flour ingredient
	2319	Food – soy flour isolate
	2320	Food – organic hi flour
	2321	Environmental – dry blending plant
	2322	Food – organic soy flour
	2323	Food – organic soy flour
	2324	Environmental – dry blending operations
	2325	Environmental – dry blending operations
	2326	Environmental – dry blending operations
	2327	Environmental – dry blending operations
	2328	Food – PIF ingredient
	2329	Food – PIF ingredient
	2330	Food – organic PDI flour
	2331	Food – PIF
	2332	Food – soy protein isolate
	2333	Food – soy protein isolate
	2334	Food – organic soy isolate
	2335	Food – organic hi PDI flour
	2336	Unknown – (food or environmental)
	2337	Unknown – (food or environmental)
	2338	Unknown – (food or environmental)
	2339	Unknown – (food or environmental)
	2340	Unknown – (food or environmental)
	2341	Unknown – (food or environmental)
	2342	Environmental – sponge

will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry (Sasser, 1997). Samples with a similarity of 0.500 or higher with a separation of 0.100 between the first and second choice are considered good library comparisons.

2.3. Statistical analysis

Values are expressed as means with their standard deviations. Differences in fatty acids among bacterial substrains were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1980). The Duncan multiple comparison method was used to differentiate among means for variables that were significantly different (Snedecor & Cochran, 1980).

Data analysis and determination of clustering were performed by principal component analysis (PCA) using Pirouette 3.11 software (InfoMetrix, Bothell, WA, USA). Each sample represents a single point in the multivariate space defined by the set of independent axes corresponding to each fatty acid concentration. Clustering in the data is observed using PCA score plots in which the data for each sample are projected onto a reduced set of principal components (PCs).

3. Results and discussion

In a previous study, it was shown that by using the GC-FID method, it was possible to differentiate the cellular fatty acid (CFA) profiles of *E. sakazakii* strains from the CFA profiles of other closely related *Enterobacter* and *Citrobacter* species (Whittaker et al., 2007). In order to evaluate the reproducibility of the GC-FID method for predicting *E. sakazakii*, the CFA profiles were determined for 30 *E. sakazakii* strains cultured on three different days. *E. sakazakii* is a Gram-negative, motile, non-sporulating, straight rod organism.

3.1. Evaluating fatty acid data for 30 E. sakazakii strains

The mean fatty acid percentages for determinations on the three different days for the 30 *E. sakazakii* strains are shown in Table 2. Fig. 1 is an example of a chromatogram for a food strain of *E. sakazaki* (sample 2322). The major fatty acids are straight chain (12:0, 14:0, 16:0) and unsaturated (16:1 and 18:1) and 17:0ω *cyclo* 7–8, all indicating a Gram-negative bacteria. The data in Table 2 show good reproducibility for the three different days and that the GC-FID analysis of FAMEs is an applicable method for identifying *E. sakazakii* isolated from food or from the environment. A summary of the average values for each fatty acid and SI for the 30 *E. sakazakii* strains is shown in Table 3. The fatty acids with the highest percentages were 16:0 with 30%, 18:1 ω7c with 24%, and 16:1 with 20% for the 90 samples. The overall predictability for the 30 samples analyzed in triplicate was high with an average SI value of 0.831.

3.2. Comparing the fatty acid profiles of E. sakazakii subgroups A and B

The subgroups for *E. sakazakii* strains were classified as A and B. Subgroups with SI values for both A and B and SI value differences of <0.1 were identified as either A/B or B/A, depending on which value was higher. Significant fatty acids 16:0, 17:0 ω *cyclo* 7–8, and 18:1 ω7c were statistically different in percentages between subgroups A and B (Table 4). A PCA model based on fatty acid profiles for substrains of *E. sakazakii* was analyzed using a scatter plot of PC1 versus PC2. The PCA scatter plot of PC1 versus PC2 for *E. sakazakii* strains classified as A, A/B, B/A, and B is shown in Fig. 2. A trend indicating class separation is observed on the PC1 axis, but the clusters are not completely separated for A/B and B/A. This was expected as the class boundaries are not distinct and assignments are not exact. The trends of A close to A/B and B close to B/A are what would be expected. However, there is a clear separation between subgroups A and B.

Fatty acid profiles of bacterial cells analyzed by GC have provided useful information for rapid detection and identification of bacteria in clinical and diagnostic bacteriology laboratories, and currently have increased significance for both food safety and food defense (Welch, 1991; Whittaker et al., 2005). Whittaker et al. (2005) have used capillary GC with FID to determine the whole cell fatty acid profiles of food-borne microbial pathogens and compared the fatty acid profiles of spores and vegetative cells of the same endospore-forming bacilli.

For isolating *E. sakazakii* in powdered milk-based infant formula, a rapid method is being developed by FDA to extract the pathogen for identification. The method involves pre-enrichment amplification in buffered peptone water for 6 h at 37 °C, centrifugation to concentrate the cell volume, plating on DFI chromogenic agar, and an overnight incubation at 37 °C. The fatty acid profile assay and real-time PCR could be used to rapidly screen infant formula samples for *E. sakazakii* and reduce the time required for the current assay by up to 5 days.

In summary, 30 *E. sakazakii* strains isolated from food or environmental sources were identified by fatty acid profiles using GC-FID. Fatty acids were extracted from whole cells, derivatized into methyl esters, and the FAMEs were identified and quantified on cultures initiated on three different days. The data in this study show that *E. sakazakii* can be identified after 24 h of growth using BHI agar with an average similarity index of 0.831 for the 30 strains. The results show that the day to day variations in percentages of fatty acids were small, and in all 90 determinations, *E. sakazakii* was predicted by using the rapid clinical library. Principal component analysis based on cellular fatty acid profiles for *E. sakazakii* strains shows separation of *E. sakazakii* subgroups A and B. This study demonstrates that the cellular fatty acid profile data using GC-FID is highly reproducible. The percentages and unique fatty acids isolated from *E. sakazakii* can provide a sensi-

Table 2
Fatty acids profiles for 30 *Enterobacter sakazakii* strains analyzed on three different days

Strain	12:0	14:0	Unknown 14.502	15:0	16:1 ω5c	16:0	17:0 ω *cyclo* 7–8	17:0	18:1 ω7c	18:1 ω5c	18:0	19:0 ω *cyclo* 8–9	Sum2[a]	Sum3[b]	SI[c]
2313	1.51 ± 0.21[d]	9.74 ± 0.12	0.80 ± 0.08	0.23 ± 0.05	0.12 ± 0.02	31.64 ± 3.14	2.22 ± 0.90	0.22 ± 0.03	22.06 ± 1.04	0.05 ± 0.04	0.53 ± 0.12	0.27 ± 0.10	10.11 ± 1.32	20.40 ± 1.72	0.787 ± 0.161
2314	1.39 ± 0.02	9.72 ± 0.47	0.74 ± 0.11	0.17 ± 0.07	0.14 ± 0.01	32.24 ± 1.66	3.02 ± 1.13	0.13 ± 0.04	22.30 ± 1.92	0.05 ± 0.05	0.46 ± 0.06	0.36 ± 0.14	9.24 ± 0.05	19.84 ± 1.62	0.853 ± 0.050
2315	1.40 ± 0.12	9.87 ± 0.16	0.76 ± 0.07	0.13 ± 0.12	0.13 ± 0.01	32.93 ± 1.25	3.33 ± 0.89	0.10 ± 0.09	21.26 ± 0.31	0.08 ± 0.01	0.52 ± 0.01	0.42 ± 0.10	9.70 ± 0.76	19.19 ± 1.17	0.814 ± 0.019
2316	1.48 ± 0.13	9.82 ± 0.46	0.77 ± 0.07	0.14 ± 0.04	0.13 ± 0.02	31.52 ± 2.31	2.68 ± 0.78	0.08 ± 0.07	22.15 ± 1.76	0.06 ± 0.05	0.52 ± 0.01	0.33 ± 0.09	9.93 ± 0.97	19.97 ± 1.08	0.813 ± 0.046
2317	1.49 ± 0.03	9.83 ± 0.56	0.79 ± 0.07	0.33 ± 0.15	0.14 ± 0.01	30.26 ± 1.83	2.54 ± 1.11	0.27 ± 0.11	23.21 ± 2.42	0.02 ± 0.04	0.43 ± 0.05	0.30 ± 0.12	10.11 ± 0.20	20.15 ± 1.71	0.808 ± 0.022
2318	1.56 ± 0.02	9.80 ± 0.16	0.77 ± 0.03	0.23 ± 0.07	0.12 ± 0.00	31.50 ± 0.15	2.97 ± 0.21	0.25 ± 0.06	22.22 ± 0.41	0.00 ± 0.00	0.55 ± 0.15	0.39 ± 0.03	10.40 ± 0.20	19.10 ± 0.70	0.824 ± 0.007
2319	1.52 ± 0.06	9.98 ± 0.28	0.75 ± 0.08	0.15 ± 0.05	0.14 ± 0.02	31.23 ± 1.51	2.80 ± 0.14	0.14 ± 0.07	21.93 ± 1.48	0.08±0.14	0.53 ± 0.07	0.41 ± 0.13	10.02 ± 0.58	20.03 ± 0.22	0.837 ± 0.035
2320	1.43 ± 0.05	9.74 ± 0.44	0.83 ± 0.14	0.30 ± 0.08	0.13 ± 0.01	31.01 ± 0.51	2.15 ± 0.58	0.29 ± 0.04	23.34 ± 1.18	0.08 ± 0.01	0.53 ± 0.06	0.26 ± 0.08	9.99 ± 0.66	19.90 ± 1.12	0.859 ± 0.069
2321	1.41 ± 0.05	9.29 ± 0.28	0.72 ± 0.01	0.25 ± 0.11	0.17 ± 0.04	29.48 ± 2.24	2.83 ± 0.24	0.28 ± 0.15	24.35 ± 1.13	0.30 ± 0.23	0.57 ± 0.04	0.53 ± 0.05	9.07 ± 0.15	19.23 ± 0.16	0.913 ± 0.039
2322	1.39 ± 0.05	9.75 ± 0.33	0.87 ± 0.03	0.46 ± 0.04	0.13 ± 0.01	31.96 ± 0.12	3.08 ± 0.45	0.43 ± 0.10	21.21 ± 1.05	0.08 ± 0.01	0.48 ± 0.04	0.36 ± 0.08	9.50 ± 0.29	20.07 ± 0.84	0.857 ± 0.035
2323	1.56 ± 0.06	9.52 ± 0.62	0.89 ± 0.17	0.36 ± 0.14	0.14 ± 0.02	29.82 ± 1.75	1.80 ± 0.81	0.49 ± 0.31	23.37 ± 2.33	0.00 ± 0.00	0.42 ± 0.06	0.15 ± 0.04	10.61 ± 0.78	20.33 ± 1.77	0.734 ± 0.133
2324	1.57 ± 0.10	9.67 ± 0.50	0.96 ± 0.16	0.25 ± 0.09	0.13 ± 0.01	30.16 ± 1.41	2.76 ± 0.80	0.22 ± 0.04	23.90 ± 1.45	0.03 ± 0.05	0.48 ± 0.08	0.41 ± 0.12	10.00 ± 0.61	19.11 ± 1.10	0.858 ± 0.099
2325	1.30 ± 0.05	9.08 ± 0.32	0.84 ± 0.11	0.42 ± 0.09	0.15 ± 0.03	31.49 ± 2.03	2.72 ± 0.40	0.45 ± 0.10	22.91 ± 1.56	0.10 ± 0.11	0.54 ± 0.03	0.36 ± 0.08	9.37 ± 0.17	19.89 ± 1.23	0.899 ± 0.047
2326	1.48 ± 0.03	9.54 ± 0.75	0.73 ± 0.06	0.12 ± 0.02	0.15 ± 0.02	31.58 ± 1.46	2.59 ± 0.47	0.08 ± 0.07	22.23 ± 1.86	0.06 ± 0.06	0.47 ± 0.05	0.34 ± 0.05	9.72 ± 0.45	20.59 ± 0.84	0.851 ± 0.007
2327	1.38 ± 0.12	9.51 ± 0.09	0.72 ± 0.02	0.14 ± 0.01	0.15 ± 0.02	31.98 ± 2.05	2.67 ± 0.77	0.12 ± 0.02	22.68 ± 1.02	0.09 ± 0.01	0.50 ± 0.08	0.36 ± 0.12	9.34 ± 0.55	20.16 ± 1.38	0.880 ± 0.044
2328	3.48 ± 0.17	6.76 ± 0.29	0.85 ± 0.08	0.28 ± 0.12	0.14 ± 0.03	30.57 ± 1.50	2.58 ± 0.59	0.30 ± 0.05	24.38 ± 1.48	0.13 ± 0.08	0.48 ± 0.03	0.33 ± 0.02	9.77 ± 0.27	19.04 ± 1.02	0.758 ± 0.046
2329	3.57 ± 0.22	6.92 ± 0.24	0.81 ± 0.08	0.25 ± 0.05	0.15 ± 0.02	30.42 ± 1.57	2.65 ± 0.46	0.24 ± 0.02	23.90 ± 1.52	0.12 ± 0.07	0.44 ± 0.05	0.32 ± 0.02	9.92 ± 0.20	19.50 ± 0.85	0.754 ± 0.040
2330	1.39 ± 0.09	9.02 ± 0.60	0.76 ± 0.04	0.23 ± 0.04	0.14 ± 0.02	31.13 ± 2.30	2.64 ± 0.49	0.27 ± 0.02	24.34 ± 1.23	0.14 ± 0.11	0.51 ± 0.02	0.42 ± 0.02	9.37 ± 0.23	18.96 ± 0.89	0.892 ± 0.027
2331	1.46 ± 0.11	9.35 ± 0.49	0.94 ± 0.34	0.21 ± 0.06	0.13 ± 0.02	31.36 ± 1.91	3.02 ± 0.91	0.22 ± 0.03	24.60 ± 2.06	0.13 ± 0.07	0.53 ± 0.06	0.45 ± 0.03	8.62 ± 0.88	18.60 ± 1.19	0.885 ± 0.071
2332	1.43 ± 0.04	9.56 ± 0.51	0.75 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	30.41 ± 1.55	2.27 ± 0.38	0.18 ± 0.07	23.48 ± 1.72	0.20 ± 0.12	0.60 ± 0.13	0.39 ± 0.05	9.15 ± 0.46	20.31 ± 0.23	0.888 ± 0.017
2333	1.42 ± 0.11	9.56 ± 0.70	0.73 ± 0.03	0.15 ± 0.06	0.14 ± 0.02	31.68 ± 1.82	2.88 ± 0.93	0.18 ± 0.09	22.78 ± 2.07	0.13 ± 0.10	0.48 ± 0.03	0.39 ± 0.05	9.15 ± 0.46	19.87 ± 1.48	0.855 ± 0.035
2334	1.47 ± 0.05	8.96 ± 0.38	0.78 ± 0.05	0.16 ± 0.04	0.14 ± 0.02	29.00 ± 2.01	2.02 ± 0.31	0.24 ± 0.09	25.35 ± 0.94	0.22 ± 0.13	0.56 ± 0.13	0.51 ± 0.25	9.34 ± 0.32	19.93 ± 0.89	0.886 ± 0.006
2335	1.43 ± 0.03	9.27 ± 0.78	0.81 ± 0.06	0.28 ± 0.10	0.13 ± 0.02	31.14 ± 1.98	2.99 ± 1.12	0.28 ± 0.07	23.86 ± 2.41	0.08 ± 0.13	0.53 ± 0.02	0.46 ± 0.10	9.26 ± 0.60	18.95 ± 1.66	0.861 ± 0.076
2336	1.64 ± 0.07	10.58 ± 0.35	0.81 ± 0.04	0.28 ± 0.13	0.16 ± 0.01	29.39 ± 0.65	2.60 ± 0.45	0.21 ± 0.09	22.01 ± 0.72	0.05 ± 0.04	0.30 ± 0.01	0.25 ± 0.04	10.11 ± 0.31	21.50 ± 0.68	0.744 ± 0.056
2337	1.32 ± 0.31	9.74 ± 0.13	0.87 ± 0.13	0.51 ± 0.46	0.18 ± 0.01	26.11 ± 0.32	2.05 ± 0.67	0.50 ± 0.41	25.05 ± 0.87	0.12 ± 0.05	0.37 ± 0.09	0.25 ± 0.06	11.12 ± 1.11	21.34 ± 1.93	0.640 ± 0.048
2338	1.32 ± 0.09	8.24 ± 0.11	0.74 ± 0.02	0.07 ± 0.06	0.17 ± 0.02	28.49 ± 2.58	0.96 ± 0.08	0.18 ± 0.07	27.94 ± 0.73	0.23 ± 0.11	0.59 ± 0.06	0.13 ± 0.13	9.12 ± 0.74	20.67 ± 0.54	0.815 ± 0.057
2339	1.14 ± 0.17	9.45 ± 0.46	0.80 ± 0.15	0.37 ± 0.28	0.19 ± 0.01	26.38 ± 1.28	1.57 ± 0.48	0.37 ± 0.24	26.05 ± 2.57	0.13 ± 0.08	0.39 ± 0.02	0.19 ± 0.01	11.18 ± 0.64	21.51 ± 0.53	0.651 ± 0.031
2340	1.25 ± 0.01	9.21 ± 0.33	0.71 ± 0.07	0.23 ± 0.10	0.16 ± 0.03	31.24 ± 1.76	2.19 ± 0.93	0.21 ± 0.06	23.46 ± 1.83	0.13 ± 0.08	0.46 ± 0.05	0.31 ± 0.03	8.30 ± 0.45	21.84 ± 1.51	0.895 ± 0.123
2341	1.40 ± 0.06	9.54 ± 0.49	0.80 ± 0.09	0.27 ± 0.13	0.15 ± 0.02	30.79 ± 2.04	2.33 ± 0.88	0.23 ± 0.08	23.94 ± 2.59	0.13 ± 0.08	0.51 ± 0.04	0.29 ± 0.06	9.32 ± 0.29	20.17 ± 1.13	0.894 ± 0.032
2342	1.37 ± 0.07	9.39 ± 0.18	0.75 ± 0.10	0.24 ± 0.08	0.15 ± 0.02	30.52 ± 2.14	2.83 ± 0.53	0.26 ± 0.02	24.59 ± 1.46	0.19 ± 0.10	0.51 ± 0.11	0.52 ± 0.02	8.56 ± 0.13	19.25 ± 1.04	0.925 ± 0.050

[a] Sum2 – 14:0 3OH/16:1 *iso*.
[b] Sum3 – 16:1 ω7c/16:1 ω6c.
[c] SI – Similarity index.
[d] Mean ± standard deviation ($n = 3$).

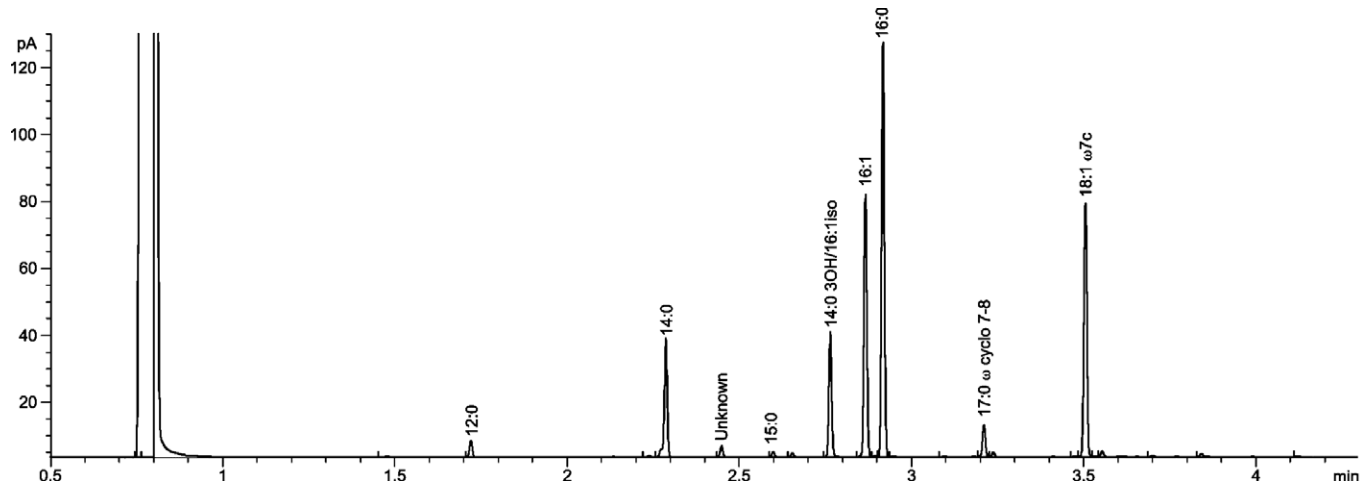

Fig. 1. Chromatogram for a food strain of *Enterobacter sakazakii* (sample 2322).

Table 3
Summary of fatty acid percentages and similarity index values for 30 strains of *Enterobacter sakazakii*

Cellular fatty acid	Fatty acid percentages
12:0	1.56 ± 0.54[a]
14:0	9.35 ± 0.86
Unknown 14.502	0.79 ± 0.11
15:0	0.24 ± 0.15
16:1 ω5c	0.15 ± 0.02
16:0	30.58 ± 2.10
17:0 ω *cyclo* 7–8	2.52 ± 0.75
17:0	0.25 ± 0.15
18:1 ω7c	23.50 ± 1.97
18:1 ω5c	0.11 ± 0.10
18:0	0.49 ± 0.09
19:0 ω *cyclo* 8–9	0.35 ± 0.13
14:0 3OH/16:1 *iso*	9.64 ± 0.82
16:1 ω7c/16:1 ω6c	19.98 ± 1.26
Similarity index	0.831 ± 0.087

[a] Values are mean ± standard deviation ($n = 90$).

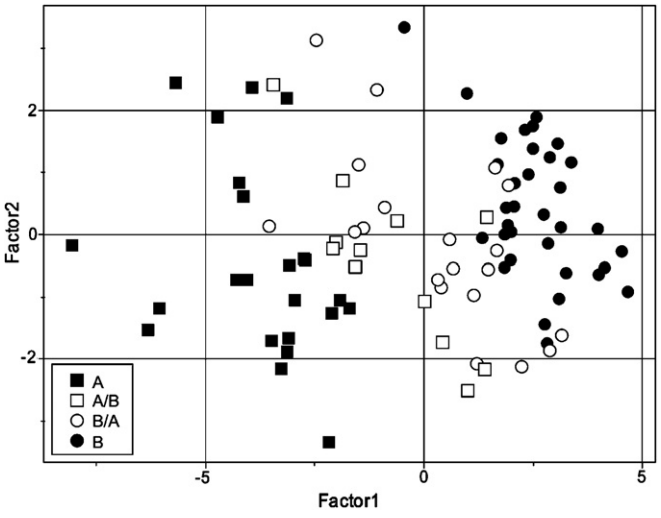

Fig. 2. Principal component analysis showing a scatter plot of PC1 vs PC2 for 90 determinations of the 30 *Enterobacter sakazakii* strains classified as A (■), A/B (□), B/A (○), and B (●).

Table 4
Summary of fatty acid percentages for *Enterobacter sakazakii* subgroups

Cellular fatty acid	*E. sakazakii* A	*E. sakazakii* B	*E. sakazakii* A/B	*E. sakazakii* B/A	*P*
12:0	1.44 ± 0.13[*a]	1.37 ± 0.17[a]	1.86 ± 0.89[b]	1.98 ± 0.91[b]	<0.001
14:0	9.69 ± 0.48[b]	9.23 ± 0.56[a,b]	8.96 ± 1.20[a]	8.84 ± 1.31[a]	0.001
Unknown 14.502	0.77 ± 0.07[a]	0.79 ± 0.11[a]	0.80 ± 0.08[a]	0.87 ± 0.19[b]	0.034
15:0	0.22 ± 0.12	0.28 ± 0.23	0.28 ± 0.09	0.22 ± 0.09	0.367
16:1 ω5c	0.14 ± 0.02[a]	0.16 ± 0.03[b]	0.14 ± 0.02[a]	0.14 ± 0.02[a]	<0.001
16:0	31.51 ± 1.65[b]	28.54 ± 2.26[a]	30.79 ± 1.53[b]	30.63 ± 1.45[b]	<0.001
17:0 ω *cyclo* 7–8	2.72 ± 0.68[b]	2.01 ± 0.76[a]	2.59 ± 0.74[b]	2.66 ± 0.66[b]	0.003
17:0	0.20 ± 0.13[a]	0.33 ± 0.22[b]	0.27 ± 0.06[a,b]	0.23 ± 0.06[a]	0.010
18:1 ω7c	22.37 ± 1.47[a]	25.24 ± 1.91[c]	23.73 ± 1.61[b]	24.09 ± 1.52[b]	<0.001
18:1 ω5c	0.08 ± 0.07[a]	0.17 ± 0.14[b]	0.09 ± 0.08[a]	0.12 ± 0.10[a,b]	0.004
18:0	0.49 ± 0.08	0.49 ± 0.11	0.49 ± 0.06	0.52 ± 0.09	0.628
19:0 ω *cyclo* 8–9	0.35 ± 0.09	0.33 ± 0.20	0.35 ± 0.10	0.42 ± 0.12	0.267
14:0 3OH/16:1 *iso*	9.59 ± 0.71	9.86 ± 1.16	9.73 ± 0.51	9.35 ± 0.79	0.359
16:1 ω7c/16:1 ω6c	20.19 ± 1.22[b]	20.32 ± 1.30[b]	19.49 ± 1.21[a,b]	19.27 ± 1.06[a]	0.029
Similarity index	0.843 ± 0.068	0.795 ± 0.127	0.835 ± 0.067	0.847 ± 0.079	0.184
n	14	7	5	4	

[*] Mean ± standard deviation. Means for a variable not sharing a common superscript 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.

tive and reproducible method for identification. This analytical method provides a rapid procedure for the identification of *E. sakazakii* strains.

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