Solid Phase Microextraction/Gas Chromatography of *Salmonella*-Infected Beef

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Eight strains of *Salmonellae* were incubated in TSB culture medium at 37 °C for 24 h. Volatile compounds derived from the bacteria were collected using solid-phase microextraction fibers and then applied to gas chromatography (GC). Similarity analysis of the GC patterns thus obtained could separate these strains on principal component similarity (PCS) scattergrams. Five major food-related pathogenic bacteria and 10 other bacteria (including one *Salmonella* strain) were also classified by growing in the same medium. It is then proposed to utilize this approach to improve the GC/PCS method of Nakai et al. [Nakai, S.; Wang, Z. H.; Dou, J.; Nakamura, S.; Ogawa, M.; Nakai, E.; Vangerstoep, J. *J. Agric. Food Chem.* **1999**, *47*, 576−583] that has been developed for screening safe foods by detecting bacteria contaminated foods. Inoculating food samples pre-enriched through preliminary incubation into a culture medium and then subjecting to the GC/PCS method after secondary incubation enhances the detectability of pathogenic bacteria.

Keywords: *GC; principal component similarity; Salmonella; beef; solid-phase microextraction*

INTRODUCTION

Typical pathogenic bacteria causing food-borne diseases are *Escherichia coli* O157:H7, *Salmonella, Vibrio parahamolyticus, Campylobacter jejuni, Listeria monocytogenes,* and *Staphylococcus aureus.* Among them, *Salmonella* in beef have frequently caused food poisoning, and especially the poisoning caused by *Salmonella enteritidis* contaminated in chicken and eggs has increased rapidly since the middle of the 1980s (Duguid and North, 1991). *Salmonellae* are anaerobic, nonspore-forming bacilli that belong to *Enterobacteriacea*. This genus has been divided into five subgenera according to their biological properties and classified into more than 2000 serovars (Krieg and Holt, 1984).

In a previous paper (Nakai et al., 1999), we reported the detection of *E. coli* and *St. aureus* in contaminated salmon and hamburger by applying principal component similarity (PCS) analysis to the gas chromotography (GC) data of volatiles derived from fish and meat samples. We have emphasized in the paper that our GC/PCS system is for screening the safe products and not for direct identification of contaminating bacteria. In the present study, we have applied the same technique to detect *Salmonella* in raw beef. However, instead of the dynamic headspace concentrator and irradiation sterilization used in the previous work (Nakai et al., 1999), solid-phase microextraction (SPME) and surface grilling of raw beef, respectively, were used for more convenience in GC analysis and construction of model contamination of the beef samples.

Although the major purpose of the GC/PCS system is quality assurance, if the method can even crudely classify contaminating bacteria, it may be helpful as a preliminary classification prior to proceeding to complete, precise identification assays. The work of accurate identification of pathogenic bacteria is usually time-consuming and thus costly and also requires a microbiologist's skill.

The objectives of this study were first to confirm the feasibility of SPME for volatile collection and grilling of raw beef for surface sterilization. Second, the capability of PCS data processing was to be tested for quick identification of contaminating bacteria when beef samples enriched by preincubation were inoculated in a test culture medium and then incubated again. This procedure is different from the previous paper (Nakai et al., 1999), in which contaminated bacteria on food samples were directly analyzed by GC without the secondary incubation.

EXPERIMENTAL PROCEDURES

SPME. Three SPME fibers were purchased from Supelco (Sigma-Aldrich Canada, Oakville, ON), namely 100 μm PDMS (polydimethyl siloxane), 65 μm PDMS/DVB (divinylbenzene), and 75 μm PDMS/Carboxen fiber. Volatiles collection from headspace and their release to GC were conducted according to Supelco's instruction manual.

Bacteria Strains and Incubation. Six *Salmonella* strains, *S. agama, S. brandenburg, S. arizonae* IIIa, *S. arizonae* IIIb, *S. meleagridis,* and *S. hader* were provided courtesy of Canadian Food Inspection Agency (Ottawa, ON). In addition, *S. enteritidis* ATCC 13070 and *S. typhimurium* ATCC 7823 were used for detection of a total of eight *Salmonella* strains. In the second analysis, *St. aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 in addition to the above last two *Salmonella* strains were used as food-related pathogenic bacteria. Also, *Klebsiella pneumoniae* ATCC 13883, *Citrobactor diversus* JCM 1658, *Klebsiella oxytoca* JCM 1665, *Serratia marcescens* ATCC 8100, *Proteus vularis* ATCC 13315, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 12228, and *Strepto-*

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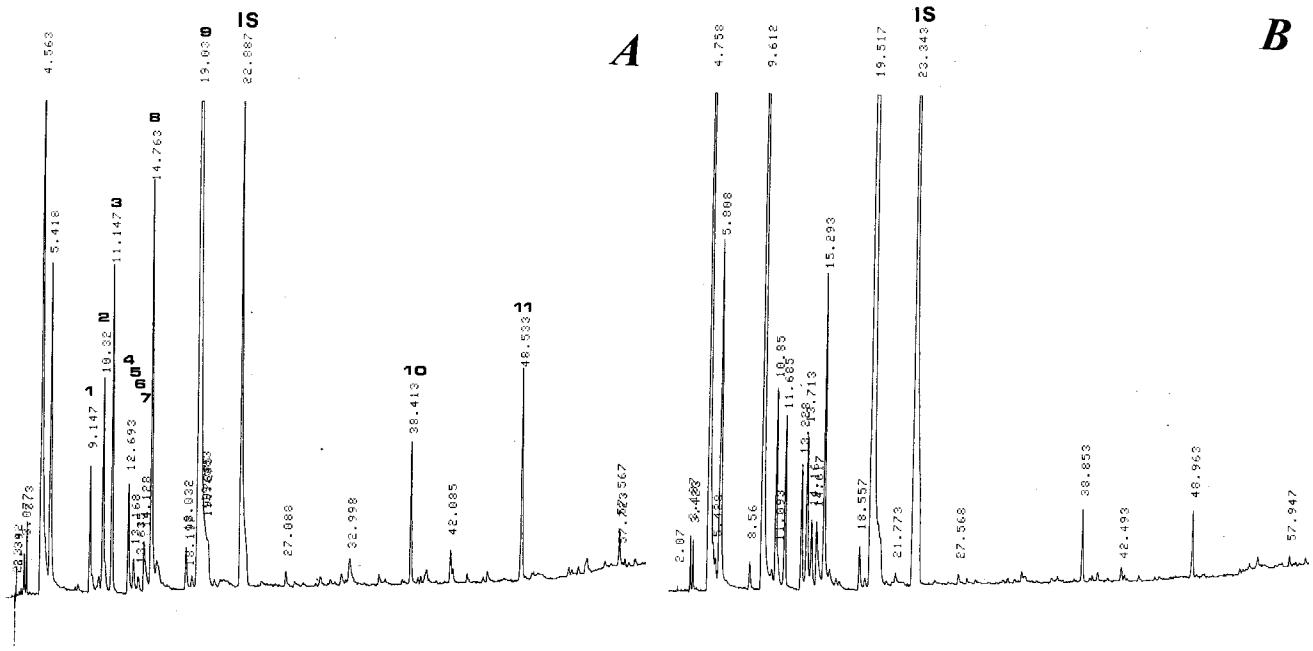

Figure 1. GC patterns of volatiles derived from *S. enteritidis* (A) and *S. typhimurium* (B), respectively, grown on TSB medium after incubation for 24 h at 37 °C. IS is the peak of internal standard.

Table 1. Peak Areas of GC Patterns of Volatiles Derived from Different Strains of *Salmonella* Grown in TSB Medium[a]

GC peak	*agama*	*brandenburg*	*arizonae* IIIa	*arizonae* IIIb	*meleagridis*	*hader*	*enteritidis*	*typhimurim*
	A	B	a	b	M	H	E	T
1	13.41 ± 8.26	14.57 ± 2.14	7.69 ± 2.00	4.79 ± 1.80	1.81 ± 0.03	2.88 ± 0.70	1.95 ± 0.89	25.89 ± 8.66
2	2.13 ± 0.78	1.95 ± 1.00	2.06 ± 0.46	2.01 ± 0.19	1.85 ± 0.35	1.96 ± 0.40	2.70 ± 0.52	0.92 ± 0.14
3	0.89 ± 1.11	0.37 ± 0.36	1.88 ± 1.56	1.71 ± 1.30	0.81 ± 0.41	1.00 ± 1.17	4.50 ± 1.02	0.68 ± 0.06
4	1.21 ± 0.49	1.43 ± 0.33	1.38 ± 0.28	0.99 ± 0.03	1.00 ± 0.17	1.02 ± 0.26	1.50 ± 0.34	0.69 ± 0.21
5	1.51 ± 1.04	3.20 ± 0.57	6.88 ± 2.15	12.12 ± 4.07	1.55 ± 0.45	1.08 ± 0.47	0.44 ± 0.10	0.92 ± 0.23
6	0.38 ± 0.31	0.97 ± 0.21	2.13 ± 0.59	1.97 ± 0.78	0.55 ± 0.16	0.46 ± 0.14	0.10 ± 0.09	0.35 ± 0.08
7	1.98 ± 1.26	0.78 ± 1.36	2.57 ± 1.07	0.66 ± 0.19	1.06 ± 0.71	1.62 ± 0.26	0.48 ± 0.43	0.34 ± 0.14
8	5.17 ± 2.02	5.66 ± 0.61	6.81 ± 0.94	6.30 ± 0.09	5.98 ± 1.33	6.40 ± 1.13	5.45 ± 1.40	2.03 ± 0.70
9	37.71 ± 22.09	75.22 ± 15.10	109.28 ± 15.39	43.72 ± 0.81	33.32 ± 6.70	41.79 ± 12.47	32.70 ± 8.40	19.27 ± 6.51
10	1.93 ± 0.88	2.97 ± 1.46	2.27 ± 0.34	2.71 ± 0.18	1.75 ± 0.44	2.30 ± 0.56	1.81 ± 0.57	0.42 ± 0.15
11	2.56 ± 1.08	3.17 ± 1.55	1.38 ± 0.20	0.98 ± 0.16	1.03 ± 0.44	0.95 ± 0.13	2.54 ± 0.84	0.37 ± 0.11

[a] The strains used were *S. agama*, *S. brandenburg*, *S. arizonae* IIIa, *S. arizonae* IIIb, *S. meleagridis*, *S. hader*, *S. enteritidis*, and *S. typhimurium*. Data are the averages of triplicate runs and their standard deviations.

coccus progenes ATCC 19615 in addition to *S. typhimurium* were used as other bacteria to be tested in the third analysis.

Bacteria was incubated in trypticase soy broth (TSB; Difco Laboratories, Detroit, MI) at 37 °C for two generations, and then 10 mL of TSB in a 15-mL vial was used for SAME extraction after incubation at 37 °C for 24 h. One microliter of ethanol solution of 0.05% valeric acid ethyl ester and 3.7 g of NaCl were added to the vials. Volatiles were collected from the headspace of the vials with a SPME fiber at 37 °C while stirring for 10 min on a heater/magnetic stirrer. Bacterial counts were taken after incubation of the cell suspension in tryptic soy agar (TSA; Difco) at 37 °C for 48 h by counting colonies grown according to the method of Swanson et al. (1992).

Mixed Culture of *E. coli* and *Salmonella*. After *E. coli* ATCC 25922 (EC) and *S. enteritidis* ATCC 13070 (SE) were incubated in tryptic soy broth (TSB), mixtures at the ratios EC (mL)/SE (mL) of 2:8, 4:6, 5:5, 6:4, 8:2, 10:0, and 0:10 were transferred into 15-mL vials. After 1 μL of ethanol solution of 0.05% valeric acid ethyl ester and 3.7 g of NaCl were added, the SPME extraction was performed.

Surface Grilling and Inoculation of Beef. After fat and fibrous tissues were removed from the sample, the surface of a beef block (10 × 20 × 10 cm) was grilled using the flame of a Bunsen burner. Then, unheated meat inside was aseptically separated and ground. To this ground beef, 10^2 CFU/mL of *S. enteritidis* or other test bacteria was inoculated and divided into 5 g each in 40-mL vials. After 25 mL of TSB culture medium was added, the vials were incubated for 24 h at 37 °C in an incubator shaker (Innova 4000 Shaker, New Brunswick Scientific, Edison, NJ) while shaking at 250 rpm. After preset time intervals, 2 μL of ethanol solution of 0.2% caproic acid ethyl ester and 15 g NaCl were added and served for SPME extraction.

GC Analysis. A model GC-9A (Shimadzu; Colombia, MD) with a medium polarity DB-624 column (30 m, 0.53 mm i.d., 3 μm film thickness; J & W Scientific, Rancho Cordova, CA) installed with FID detector (250 °C) was used. Flow rates (mL/min) were air, 500; H_2, 50; and makeup He, 50. The column temperature was held at 35 °C for 5 min, ramped to 200 °C at 3 °C/min, and then to 220 °C at 10 °C/min and held for 20 min. Carrier gas was He. Thermal desorption of volatiles from the SPME fiber was carried out in the injection port at 220 °C in the splitless mode by holding for 5 min. A Chromatopac C-R3A (Shimadzu, Tokyo) connected with a PC computer was used to monitor GC patterns.

Data Processing. Data processing was conducted as described in the previous paper (Nakai et al., 1999).

RESULTS

SPME Conditions. An extraction time of 10 min in the presence of 25% NaCl from *S. enteritidis* grown in the TSB medium for 24 h at 37 °C ($1.1-1.9 \times 10^9$ CFU/mL) collected the maximum amount of volatile com-

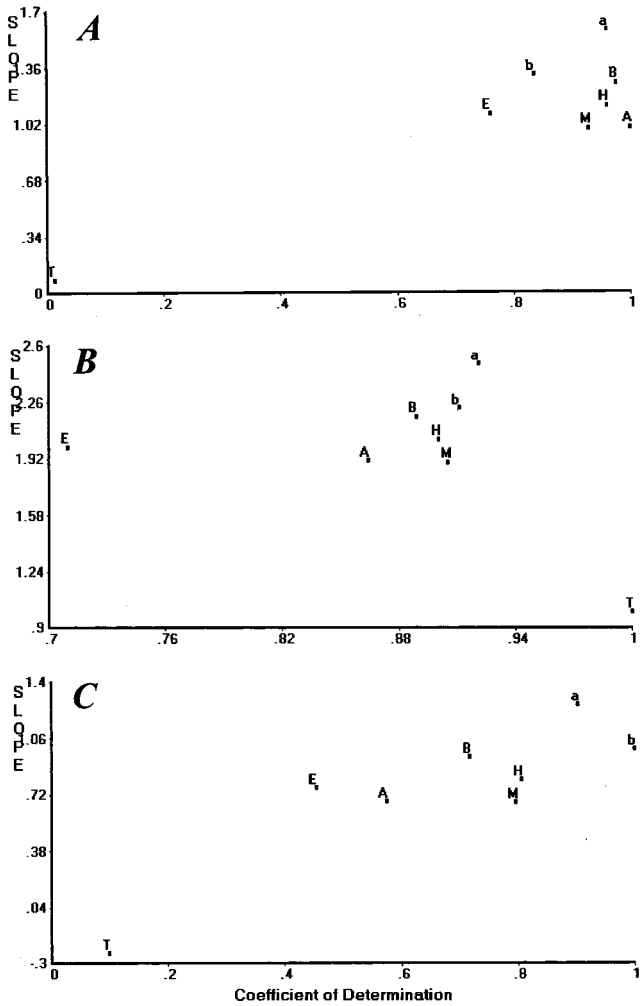

Figure 2. PCS scattergrams of *Salmonella* strains. A, *S. agama*; B, *S. brandenburg*; a, *S. arizonae* IIIa; b, *S. arizonae* IIIb; M, *S. meleagridis*; H, *S. hader*; E, *S. enteritidis* ATCC 13070; and T, *S. typhimurium* ATCC 7823. For panels A, B, and C, strains A, T, and b were used as the references, respectively. Each data point is the average of triplicate GC runs as shown in Table 1.

pounds. Three fibers, PDMS, PDMS/DVB, and PDMS/Carboxen, showed the total relative peak area of 6031, 26 989, and 128 407, respectively, on the C-R3A Chromatopack. Accordingly, 75 μm PDMS/Carboxen was subsequently used throughout this study.

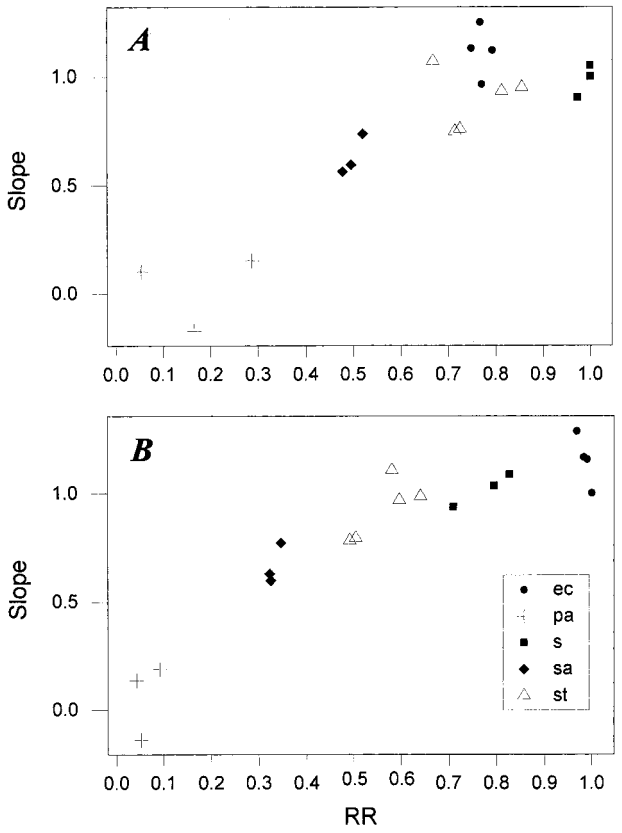

Figure 3. PCS scattergram of food-related pathogenic bacteria. ec, *E. coli*; pa, *P. aeruginosa*; s, *S. enteritidis*; sa, *St. aureus*; st, *S. typhimurium*. (A) s2 used as the reference and (B) ec4 used as the reference.

***Salmonella* Strains.** Figure 1A,B show GC patterns of *S. enteritidis* (1.5×10^9 CFU/mL) and *S. typhimurium* (7.0×10^9 CFU/mL), respectively, after incubation for 24 h at 37 °C in the TSB medium. Table 1 indicates the averages of peak areas from triplicate runs, one GC each of three samples per *Salmonella* strain. Figure 2A−C shows PCS scattergrams when strains A (*S. agama*), T (*S. typhimurium*), and b (*S. arizonae* IIIb) were used as the references, respectively. Facile separation of data points for T and E (*S. enteritidis)* is observed in Figure 2A,B. Figure 2C shows the best separation of all strains, except for strains H (*S. hader*) and M (*S. meleagridis*). Further rotation of the reference did not improve the separation of strains H and M more than that in Figure

Table 2. Peak Areas of GC Patterns of Volatiles Derived from Other Bacteria Grown in TSB Medium[a]

GC peak	*enteritidis*	*pneumoniae*	*diversus*	*oxytoca*	*marcescens*	*vulgaris*	*subtilis*	*luteus*	*epidermidis*	*pyogenes*
	E	P	D	O	M	V	S	L	e	p
1	0	0.20 ± 0.11	0.14 ± 0.03	0	1.26 ± 0.40	0	0	0.24 ± 0.21	1.07 ± 0.18	4.02 ± 2.48
2	2.28 ± 1.46	0.86 ± 0.11	34.20 ± 5.76	2.59 ± 1.84	0.93 ± 0.03	0.45 ± 0.03	0.08 ± 0.13	0	0.70 ± 0.06	0.58 ± 0.27
3	2.70 ± 0.52	2.47 ± 1.23	3.14 ± 0.63	9.47 ± 3.14	2.87 ± 1.39	1.68 ± 0.21	4.07 ± 0.45	5.85 ± 0.91	2.12 ± 0.40	2.33 ± 0.82
4	4.50 ± 1.02	0.70 ± 0.30	2.77 ± 0.71	0.75 ± 0.54	1.04 ± 1.18	0.54 ± 0.82	2.50 ± 1.40	0.65 ± 0.73	0.74 ± 0.81	1.86 ± 1.63
5	1.50 ± 0.34	0.85 ± 0.41	2.14 ± 0.20	0.96 ± 0.54	0.60 ± 0.26	0.52 ± 0.34	0.07 ± 0.13	0	0.45 ± 0.09	0
6	0.25 ± 0.22	2.13 ± 1.02	0.54 ± 0.42	1.73 ± 0.64	7.40 ± 1.31	0	0	3.71 ± 1.71	7.55 ± 1.67	21.35 ± 5.68
7	0.10 ± 0.09	0.47 ± 0.26	0.46 ± 0.13	0.49 ± 0.08	1.27 ± 1.10	1.51 ± 0.31	0	0	1.98 ± 0.60	7.58 ± 2.40
8	0.66 ± 0.14	1.98 ± 1.30	1.17 ± 0.67	0.72 ± 0.98	4.76 ± 0.86	0.44 ± 0.07	0	0	6.39 ± 1.98	0.81 ± 0.15
9	5.45 ± 1.40	3.92 ± 2.31	6.17 ± 0.83	5.75 ± 2.38	5.07 ± 0.40	0.74 ± 0.14	5.67 ± 1.27	0	4.25 ± 0.77	2.85 ± 0.71
10	0.49 ± 0.00	0.95 ± 0.23	20.97 ± 8.05	2.19 ± 0.68	2.71 ± 0.42	0	4.86 ± 0.88	0	1.51 ± 0.56	0.92 ± 0.14
11	32.70 ± 8.40	18.14 ± 9.65	24.37 ± 3.66	58.40 ± 19.87	14.95 ± 1.36	5.08 ± 0.47	11.06 ± 2.25	4.10 ± 0.38	11.60 ± 3.55	0.38 ± 0.28
12	0.07 ± 0.13	0.15 ± 0.28	1.47 ± 0.41	0.35 ± 0.60	1.78 ± 0.75	0.16 ± 0.16	0	2.37 ± 1.04	1.52 ± 0.48	6.61 ± 0.18
13	1.81 ± 0.57	3.70 ± 1.94	3.65 ± 0.48	1.40 ± 1.09	14.64 ± 0.44	8.06 ± 0.50	0.17 ± 0.04	0.18 ± 0.16	14.66 ± 2.60	0.93 ± 0.57
14	2.54 ± 0.84	2.21 ± 2.14	4.87 ± 0.53	0.61 ± 0.16	12.44 ± 1.06	2.70 ± 0.62	0.24 ± 0.04	0.34 ± 0.11	2.69 ± 0.28	0.20 ± 0.10
15	0	6.26 ± 5.52	26.68 ± 6.27	20.20 ± 2.12	0.06 ± 0.10	1.84 ± 0.69	1.11 ± 0.20	0	0.06 ± 0.11	0

[a]The bacteria used were *K. pneumoniae*, *C. diversus*, *K. oxytoca*, *S. marcescens*, *P. vulgaris*, *B. subtilis*, *M. luteus*, *St. epiderimidis*, and *S. pyogenes*. Data are the averages of triplicate runs and their standard deviations.

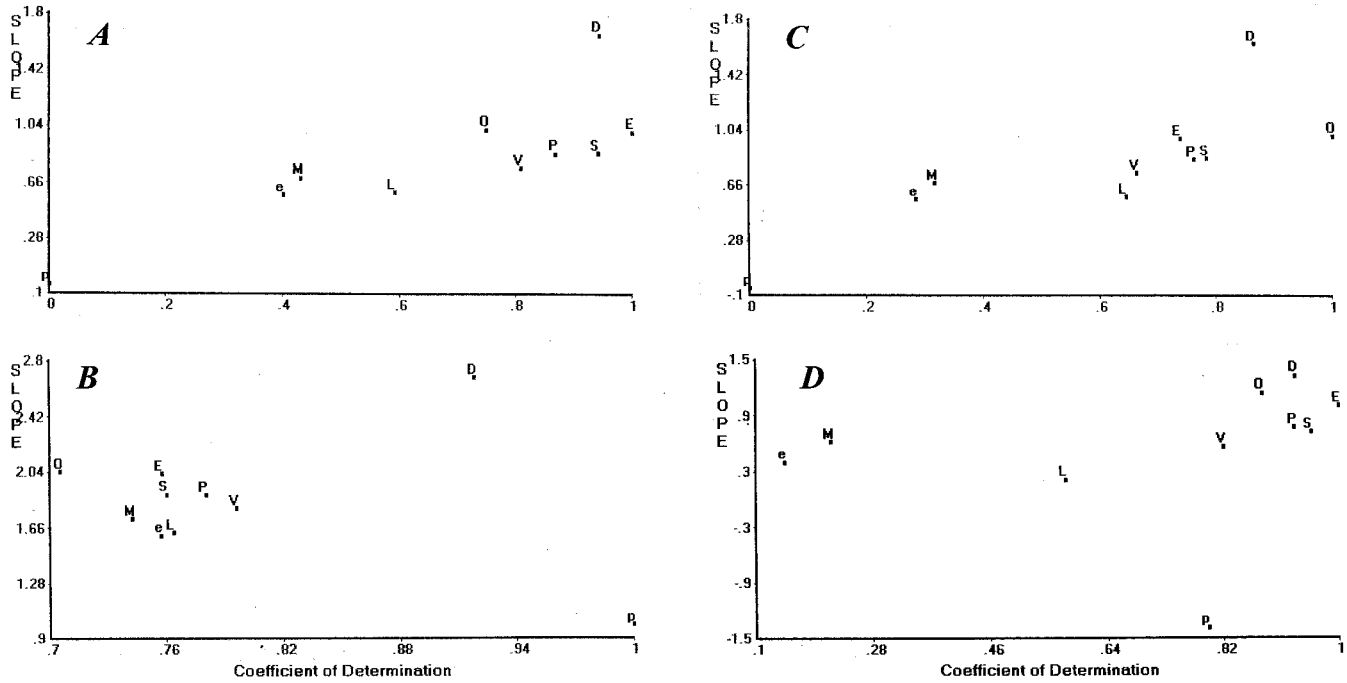

Figure 4. PCS scattergrams of other bacteria. P, *K. pneumoniae*; D, *C. diversus*; O, *K. oxytoca*; M, *Ser. marcescens*; V, *P. vulgaris*; S, *B. subtilis*; L, *M. luteus*; e, *St. epidermidis*; P, *Str. pyogenes*; and E, *S. enteritidis* used for comparison. For panels A, B, and C, bacteria E, P, and O were used as the references. For panel D, three PC scores were used for PCS computation instead of five PC scores in computation for other scattergrams. Each data point is the average of triplicate GC runs as shown in Table 2.

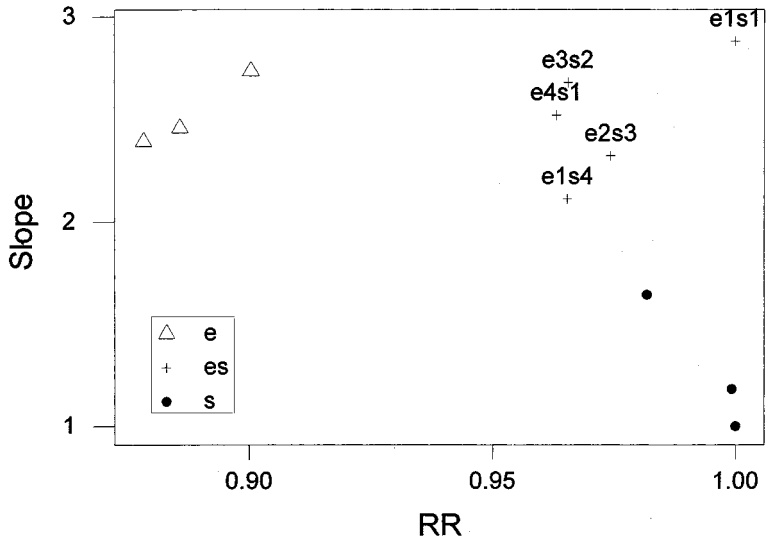

Figure 5. PCS scattergram of the mixtures (es) after incubation of *S. enteritidis* (s) and *E. coli* (e) on TBS medium for 24 h at 37 °C. Digits attached to signs e and s are mixing ratios. For instance, e4s1 is the e−s mixture at the ratio of 4:1.

2C, meaning that quite similar GC patterns were observed for those two strains. *S. enteritidis* and *S. typhimurium* were characteristic, with large peaks 3 and 1, respectively (Figure 1).

Food-Related Pathogenic Bacteria. *S. enteritidis*, *S. typhimurium*, *St. aureus*, *Pseudomonas aeruginosa*, and *E. coli* were chosen as representative food-related bacteria. PCS scattergrams from triplicate runs of all bacteria are shown in Figure 3 when *S. enteritidis* (Figure 3A) and *E. coli* (Figure 3B) were used as the references. *Pseudomonas aeruginosa* (pa) and *St. aureus* (sa) are well separated from *S. enteritidis* (s), while *S. typhimurium* (st) and *E. coli* (ec) locate nearby each other in Figure 3A. However, as shown in Figure 3B, the latter two bacteria are better separated by rotating the reference from s to ec. Unlike Figure 2 using the averages of triplicate runs, this scattergram shows all three data points of triplicate runs for each bacterium, thus demonstrating the repeatability of the GC/PCS runs.

Other Bacteria. Gram-negative bacteria were represented by *S. enteritidis*, *Klebsiella pneumoniae*, *Citrobacter diversus*, *Klebsiella oxytoca*, and *Serratia marcescens*, whereas *Proteus vulgaris*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* were chosen as Gram-positive bacteria. In a fashion similar to Figure 2, averages of triplicate runs were used for PCS computation (Table 2). Figure 4A−C shows PCS scattergrams when *S. enteritidis* (E), *Str. pyogenes* (p), and *K. oxytoca* (O), respectively, were used as the references. The separation of some bacteria appears to be difficult, such as *K.*

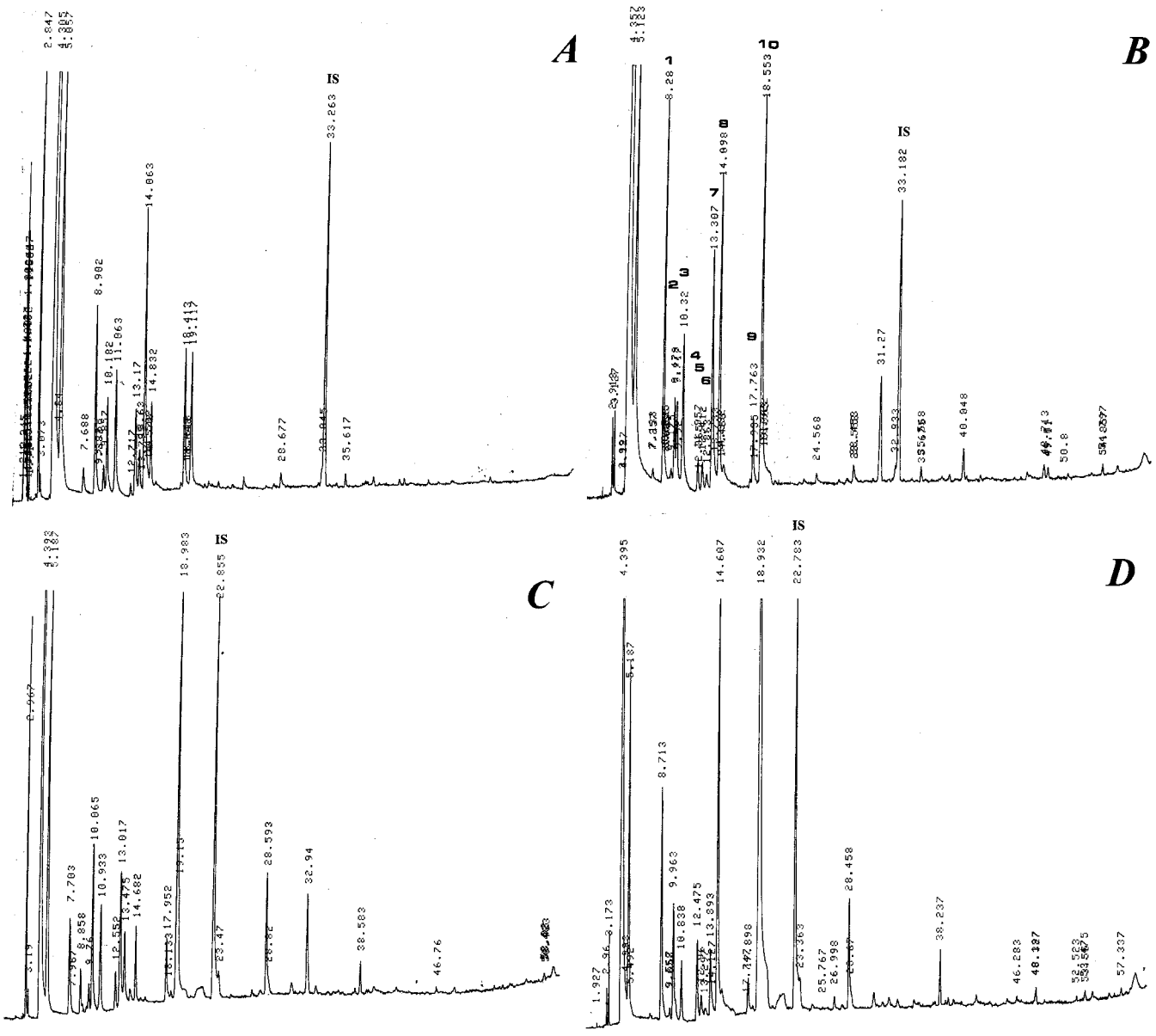

Figure 6. GC patterns of volatiles derived from *S. enteritidis* inoculated on surface sterilized raw beef and incubated for 9 h (A) and 24 h (B) at 37 °C. Panels C and D are *S. enteritidis* grown in the TSB medium for comparison. IS is the peak of internal standard.

pneumoniae (P), *P. vulgaris* (V), and *B. subtilis* (S) from E. Rotating the reference for drawing the PCS scattergram did not further improve the separation in this case. The best separation of V from P and S was achieved when only three PC scores were used for PCS computation (Figure 4D), while five PC scores were used in all other computations. Changing the number of PC scores used for PCS computation did not improve the separation as much as rotating the reference. The separation of these bacteria was almost satisfactory although it was not fully successful.

Mixed Cultures after Incubation of *S. enteritidis* and *E. coli*. Figure 5 is the plot of all three data points from triplicate runs of two bacteria except for their mixture with a single data point for each mixing ratio. It is apparent that the mixed cultures appear apart from their pure cultures. After a long incubation, there was a trend that data points approached to that of the dominating species as reported by Horimoto et al. (1997) in milk.

***Salmonella* Inoculated in Beef.** Figure 6A,B shows GC patterns after incubating *S. enteritidis* in raw beef at 37 °C for 9 h (2.0×10^8 CFU/mL) and 24 h (1.7×10^9 CFU/mL), whereas Figure 6C,D shows *S. enteritidis* grown on the TSB medium after incubating at the same temperature for 9 h (2.9×10^8 CFU/mL) and 24 h (1.0×10^9 CFU/mL), respectively, which were used for comparison with the growth in meat. Peaks 1−10 (Figure 6B) were used for PCS computation.

Figure 7 shows PCS scattergrams based on GC patterns after incubating for 0, 6, 9, 15, and 24 h at 37 °C. *Salmonella* grown in beef (b) was compared with the same bacteria grown in the TSB medium (m). Bacterial growths were about the same between the two samples as shown in *Salmonella* counts of 2.8×10^6, 2.0×10^8, 1.4×10^9, and 1.7×10^9 CFU/mL in beef as compared to 6.1×10^5, 2.9×10^8, 1.1×10^9, and 1.0×10^9 CFU/mL in the TSB medium after 6, 9, 15, and 24 h incubation, respectively. Figure 7A using 0 h beef as

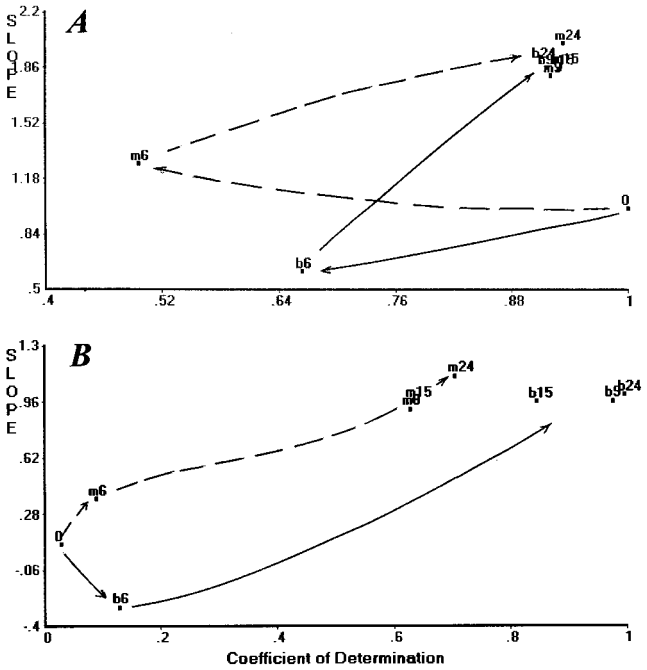

Figure 7. PCS scattergram of *S. enteritidis* grown on beef (b) and TSB medium (m). Digits after b and m show the incubation hours at 37 °C. References used were 0 h sample (A) and sample incubated in beef for 24 h (B).

the reference indicates highly similar growth patterns between the beef (b6−b24) and the medium (m6−m24) except for the volatiles pattern of 6 h incubation (m6 vs b6). This difference may have been due to a change in endogenous volatile compounds included in the beef and the culture medium, which have dissipated during incubation thereafter. When 24 h incubated beef (b24) was used as the reference (Figure 7B), patterns from the samples grown in beef and broth could be easily distinguished. It may, therefore, be possible to conclude that the patterns of volatiles produced by bacteria when grown in beef and TSB medium are rather similar.

DISCUSSION

In the previous paper (Nakai et al., 1999), the GC/PCS system was used to ensure food safety by certifying whether samples showed absence of vapors from either contaminating bacteria or chemically derived off-flavor compounds. The main purpose at that time was not to identify the contaminating bacteria but to ensure the absence of pathogenic bacteria. However, if some further identification can be achieved, even if it may be a crude separation, it should assist the subsequent complete, precise confirmation assays to identify infecting bacteria that cause spoilage or food poisoning. After GC/PCS is carried out on preliminary incubated samples, the secondary incubation can be made on the culture media after inoculating the pre-enriched food sample. More accurate identification can then be expected than single GC/PCS work when food samples are incubated only once.

When insufficient separation on PCS scattergrams arises such as between *K. pneumoniae*, *P. vulgaris*, and *B. subtilis* as shown in Figure 4, (1) the rotation of reference for PCS computation should be tried first as evidenced in Nakai et al. (2000) and (2) the reduction of the number of PC scores being employed in the PCS computation should be also tried as shown in Figure 4D although it may be less effective. The idea behind this second treatment is to utilize greater differences in the slopes within smaller numbers of PC scores in the linear regression of slope on the proportion of variability, which the PC scores can account for. When none of these methods are effective, changing the culture media may be required. Figure 7 in this study illustrates the improved distinction of bacterial strains by following pattern change during incubation. Addition of any other parameters characterizing bacteria species into the PCS computation, even if it is not GC data would be useful in distinguishing very difficult classification such as *Salmonella* strains H and M as shown in Figure 2.

An advantage of the new method proposed in this study is that once the data on bacteria grown in a culture media is established, it can be applied to any foods when the food samples incubated for pre-enriching bacteria is inoculated into the specific culture medium and then incubated again in that selected medium. If specific bacterial species are of concern, such as *Salmonellae*, its selective media as reported by Tan and Shelef (1999) should be used. Inoculation of the bacteria into sterilized foods as was used in the previous paper (Nakai et al., 1999) is no longer required for identification of contaminating bacteria. This new approach will also avoid the need of sterilization of food samples for constructing the model inoculation GC patterns, which is tedious and sometimes complicated by causing lipid oxidation in the food samples so as to interfere with the GC analysis, as reported for hamburger samples (Nakai et al., 1999). This new approach, however, cannot substitute for the previous method (Nakai et al., 1999) when rapid detection of bacterial contamination is required without further proceeding to the secondary incubation in the culture medium. Another advantage of the previous method is the need of a professional microbiologist's expertise to a lesser extent. It also may be optional to inoculate heavily contaminated samples directly without further incubating into a selective media as discussed by Tan and Shelef (1999).

In conclusion, this study has demonstrated the feasibility of improving the detectability of food samples contaminated with different bacterial strains by growing the bacteria in the samples by incubating them into a culture medium rather than by analyzing the incubated food samples directly without the secondary incubation, as suggested in the previous method (Nakai et al., 1999).

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