Analysis of Cellular Fatty Acid Methyl Esters (FAMEs) for the Identification of *Leuconostoc* Strains Isolated from Kimchi

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The cellular fatty acid methyl esters (FAMEs) analysis data obtained for clusters defined at a Euclidian distance of 17.5, in the classification of lactic acid bacteria isolated from kimchi, described by Lee *et al.* (4), was used for the identification of 79 *Leuconostoc* isolates. The test strains were isolated using a selective isolation medium specific for the genus *Leuconostoc*. These strains were then characterized according to their fatty acid profiles. The results show that all seventy nine test strains were identified to the known *Leuconostoc* clusters B, C and D. Cluster B had the highest relative amount of the saturated fatty acid 16:0. The saturated fatty acid 16:0 and summed feature 9 were found as a major components in cluster C, which had a higher level of summed feature 9 than cluster B. Cluster D is characterized by the highest relative amount of the unsaturated fatty acid 18:1 w9c. It is suggested that FAMEs analysis can be successfully applied in the identification of lactic acid bacteria isolated from kimchi.

Key words: Cellular fatty acid profiles, lactic acid bacteria, chemotaxonomy

Kimchi is a generic term used to denote a group of fermented vegetable foods found in Korea. The flavor of kimchi is dependent on the ingredients, fermentation conditions, e.g. temperature, and the bacteria involved in the fermentation process (1, 3, 5). In particular, the genera *Lactobacillus, Leuconostoc and Pediococcus* are known to play an important role in kimchi fermentations (1, 3, 5). The genera *Lactobacillus, Leuconostoc* and Pediococcus have similar physiological and biochemical characteristics (2). Phylogenetically, these three genera are considered intermixed (9, 11). Therefore, there is a strong need to determine the taxonomic status of these genera to aid rapid classification and identification in the future.

Fatty acids are an important structural constituent of the cell envelope. Cellular fatty acid composition, as analyzed by gas-liquid chromatography, has long been recognized as a valuable chemotaxonomic tool for the clas-

sification and identification of bacteria (6, 10). Recently, an automated, computer interfaced, gas-liquid chromatography system was introduced for the identification of bacteria on the basis of their cellular fatty acid profiles (12).

In a previous paper (4), we constructed a database of FAMEs fingerprints by using the Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Delaware, U.S.A.). The database comprised data derived from 230 lactic acid bacteria isolated from kimchi, including 19 type strains. The database composed 7 major clusters and 1 single member cluster defined at a Euclidian distance of 17.5. These aggregate taxa were equivalent to the genus *Leuconostoc* (aggregate group A, B, C and D), the genus *Lactobacillus* (aggregate group F), the genera *Lactobacillus* and *Pediococcus* (aggregate group E) and the genera *Leuconostoc* and *Lactobacillus* (aggregate group G).

In this study, we used this database for the rapid identification of 79 presumptive *Leuconostoc* strains isolated from kimchi.

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Table 1. 79 *Leuconostoc* isolates identified to Know clusters B, C and D

Cluster	Strain (Lu)
B	4, 5, 9, 13, 14, 28, 31, 32, 38, 42, 50, 55, 56, 61, 62, 68, 87, 88, 92, 94, 95. 100, 102, 109, 124, 129, 130, 134, 148, 153, 155, 157, 161, 161-1, 167, 177, 186, 187, 188, 192, 196, 205, 210, 251, 252, 253, 267, 275, 284, 290
C	10, 19, 29, 63, 91, 96, 97, 103, 104, 108, 113, 117, 227, 559
D	7, 15, 53, 110, 112, 114, 133, 133-1, 137, 141, 143, 149, 165, 200

Table 2. Major fatty acid profiles of test strains[a]

Compound	Frequency (%)	% in lactic acid bacteria		
		Mean[b] (%)	Minimum value	Maximum value
Saturated fatty acids				
12:0	25	0.07	0.00	0.38
14:0	100	8.33	1.00	19.85
15:0	73	0.26	0.00	0.58
16:0	100	24.39	2.34	35.14
18:0	100	1.24	0.63	2.55
20:0	15	0.10	0	1.85
Unsaturated fatty acids[c]				
16:1 w9*c*	24	0.05	0.00	0.36
16:1 w5*c*	86	0.46	0.00	0.77
17:1 w8*c*	95	0.54	0.00	1.00
18:1 w9*c*	100	18.30	1.34	44.45
20:2 w6, 9*c*	11	0.05	0	0.55
Branched fatty acids				
15:0 *iso*	9	0.02	0.00	0.28
15:0 *anteiso*	32	0.08	0.00	0.42
16:0 *iso*	35	0.01	0.00	0.20
17:0 *anteiso*	61	0.24	0.00	0.72
19:0 *iso*	90	0.69	0.00	1.80
Hydroxy				
16:0 2OH	2	0.12	0.00	1.90
17:0 *iso* 3OH	14	0.13	0.00	1.84
Cyclopropane				
17:0 CYCLO	49	0.38	0.00	3.25
19:0 CYCLO w8*c*	94	11.29	0.00	19.89
Summed features[d]				
Summed feature 1				
Summed feature 4				
Summed feature 6				
	85	0.47	0.00	1.90
Summed feature 7				
	100	8.59	1.12	17.36
Summed feature 9				
	95	8.43	0.00	29.24

[a] Fatty acids 20:1 w9*t*, 20:1 w9*c*, and summed feature 5 were present in less than 5% of the strains tested.

[b] Mean values were calculated by using FAME data for the tested strains, regardless of the fact that every fatty acid was not detected in all of the strains.

[c] The position of the double bond can be located by counting from methyl (w) end of the carbon chain. A *cis* isomer is indicated by the suffix *c*.

[d] Summed features represent groups of two or three fatty acids which could not be separated by gas-liquid chromatography with the MIDI system. Summed feature 1 contained one more of following fatty acids: 14 : 1 w5*t* and/or 14 : 1 w5*c*. Summed feature 4 contained one more of following fatty acids: 15 : 0 *iso* 2OH and/or 16 : w1 7*t*. Summed feature 6 contained one or more of following fatty acids: 18 : 0 *anteiso* and/or 18 : 2 w6, 9*c*. Summed feature 7 contained one or more of following fatty acids: 18 : 1 w7*c*, 18 : 1 w9*t* and/or 18 : 1 w12*t*. Summed feature 9 contained one or more of following fatty acids: unknown 18.846, unknown 18.858 and/or 19 : 0 CYCLO w10*c* (*cis* isomer is indicated by the suffixes *c*).

Materials and Methods

Strains

All of the test strains were isolated from kimchi using a *Leuconostoc* specific selective isolation medium, i.e. phenylethyl alcohol sucrose (PES) medium. The composition of the medium was; tryticase peptone 5 g, yeast extract 0.5 g, sucrose 2 g, $(NH_4)_2SO_4$ 2 g, $MgSO_4 \cdot 7H_2O$ 0. 5 g, KH_2PO_4 1 g, phenylethyl alcohol 2.5 ml, agar 15 g, distilled water 1 liter, pH 6.8 (7). Seventy nine presumptive *Leuconostoc* strains were isolated and are listed in Table 1. The test strains were subcultured onto MRS medium and incubated at 30°C for 48 hours for cellular fatty acid analysis (4).

Fatty acid methyl esters (FAMEs) analysis

FAMEs were prepared and extracted according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Delaware, U.S.A.). The extracts were analyzed with a Hewlett-Packard model HP5890A gas chromatograph equipped with a 25 m×0.2 mm methyl phenyl silicone fused silica capillary column (HP 19091B-102). The details of the chromatography conditions are as follows: carrier gas, hydrogen; column head pressure, 10 psi; split ratio, 100:1; split vent, 50 ml/min; septum purge, 5 ml/min; FID hydrogen, 30 ml/min; FID nitrogen, 30 ml/min; FID air, 400 ml/min; initial temperature, 170°C; program rate, 5°C/ min; final temperature, 270°C; FID temperature, 300°C; injection port, 250°C; injection volume, 2 μl. FAMEs profiles were analyzed by Microbial Identification System software (MIDI; Microbial ID, Inc., Newark, Delaware, U.S.A.). The identification of the test strains was achieved by comparison with a database described elsewhere(4).

Results and Discussion

Fatty acid composition of the 79 presumptive *Leuconostoc strains*

The fatty acid compositions of the 79 presumptive *Leuconostoc* strains were determined. Three of the 28 different fatty acid types were excluded from the final data analysis since these were detected in less than 5% of the strains. The frequencies and mean percentages of the other 25 FAMEs are shown in Table 2.

All 79 strains contained 16:0, 18:0, 18:1 w9c, summed feature 4 (15 *iso* 2OH and/or 16:1 w7t) and summed feature 7 (18:1 w7c, 18:1w 9t and/or 18:1 w12t). Seven fatty acids appeared in more than 70% of the strains tested (15:0, 16:1 w5c, 17:1 w8c, 19:0 *iso*, 19:0 CYCLO 8c, summed feature 6 (18:0 *anteiso* and/or 18:2 w6,9c) and summed feature 9 (unknown 18.846, unk-

nown 18.858 and/or 19:0 CYCLO w10c)).

Identification of the 79 presumptive *Leuconostoc* strains

Test strains were identified by comparison with the database described by Lee *et al.* (4) using Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Delaware, U.S.A.). All 79 strains were identified to known clusters at a Euclidian distance of 17.5. The fatty acid

Table 3. Major cellular fatty acid composition of *Leuconostoc* isolates identified to known clustes B, C and D

Compound[a]	% (Mean percentage of total)		
	cluster B	cluster C	cluster D
Saturated fatty acids			
12:0	tr	tr	tr
14:0	7.8(1.4)	9.2(4.3)	11.1(2.9)
15:0	tr	tr	tr
16:0	31.4(2.0)	22.0(4.8)	23.4(2.8)
18:0	1.0(2.0)	1.8(0.6)	1.2(0.3)
20:0	tr	tr	ND
Unsaturated fatty acids			
16:1 w9c	tr	tr	tr
16:1 w5c	tr	tr	tr
17:1 w8c	tr	tr	0.8(0.1)
18:1 w9c	17.3(3.1)	14.0(4.0)	31.9(6.1)
20:2 w6, 9c	tr	tr	tr
Branched fatty acids			
15:0 *iso*	tr	tr	tr
15:0 *anteiso*	tr	tr	tr
16:0 *iso*	ND	tr	tr
17:0 *anteiso*	tr		
19:0 *iso*	0.7(0.3)	tr	tr
Hydroxy			
16:0 2OH	tr	tr	tr
17:0 *iso* 3OH	tr	tr	tr
Cyclopropane			
17:0 CYCLO	tr	1.3(1.2)	tr
19:0 CYCLO w8c	14.1(3.6)	10.5(4.8)	4.6(3.3)
Summed features			
Summed feature 1			
	ND	tr	tr
Summed feature 4			
	8.8(1.1)	8.6(3.2)	8.4(1.4)
Summed feature 6			
	tr	tr	0.6(0.4)
Summed feature 7			
	11.1(2.2)	6.5(4.0)	5.9(2.2)
Summed feature 9			
	5.2(1.9)	22.1(4.0)	8.1(5.2)

[a] The definition of the fatty acids is the same as explained in the footnotes of Table 2.
[b] Number of strains within the cluster.
( ) standard deviation.
[d] tr, trace (<0.5%).
[e] ND, not detected.

mean percentages and standard deviations of these clust-ers are presented in Table 3.

Cluster B (*Leuconostoc* sp.) consisted of 51 strains. The members of this cluster contained the highest relative amount of the saturated fatty acid 16 : 0.

Cluster C (*Leuconostoc* sp.) included 14 strains and had a higher level of summed feature 9 than cluster B.

Cluster D (*Leuconostoc* sp.) was formed by 14 strains and is characterized by the highest relative amounts of the unsaturated fatty acid 18 : 1 w9c.

It is concluded that the cellular FAMEs analysis tech-nique can success fully be used for the identification of presumptive *Leuconostoc* strains isolated from kimchi. It is suggested that this method can be applied for the ra-pid identification of lactic acid bacteria.

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