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Classification of Isolates Originating from Kimchi Using Carbon-source Utilization Patterns

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One hundred and eighty two lactic acid bacteria, isolated mainly from kimchi, including reference strains were examined for their ability to utilize 95 carbon sources. The test strains were assigned to 5 major, 1 minor and 12 single-membered clusters based on the S_{SM}, UPGMA algorithm (at similarity of 80%). These aggregate clusters were equivalent to the genus *Leuconostoc* (aggregate cluster M and N), the genus *Lactobacillus* (aggregate cluster Q and R), and the genera *Lactobacillus* and *Leuconostoc* (aggregate cluster O and P) according to the database of the Biolog system. This study demonstrates that rapid identification and classification of isolates originating from kimchi can be achieved on the basis of such carbon source utilization tests.

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 (2, 3, 11, 12). In particular, the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* are known to play an important role in the fermentation of kimchi (2, 3, 11, 12). The genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* have similar physiological and biochemical characteristics (9). Phylogenetically, these three genera are considered intermixed (15, 19). Therefore, there is a real need to determine the taxonomic status of these genera to aid rapid classification and identification in the future.

The Biolog system (Biolog, Inc., Hayward, CA, U.S.A.) is an automated identification and classification system for microorganisms. The system is designed to detect the utilization pattern of a test strain with regard to 95 different carbon sources which include amino acids, carboxylic acids, and carbohydrates. The resultant metabolic profile of the test strain is compared with those of strains in a database. With relevance to this study is the 'Microlog computer software version 3.50' which contains data on 57 species of lactic acid bacteria (1, 10, 16, 17).

In this study, 167 isolates from kimchi and 15 representative strains of the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* were investigated using the Biolog system and subsequently classified.

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MATERIALS AND METHODS

Strains

All of the isolates from kimchi were obtained from the Cellular Response Modifier Research Unit, Korea Research Institute of Bioscience and Biotechnology (KRIBB), KIST, Taejon, Korea (3). The reference strains were obtained from the Korean Collection for Type Cultures (KCTC), the Korea Research Institute of Bioscience and Biotechnology (KRIBB), KIST, Taejon, Korea. Designations and sources of test strains are shown in Table 1.

Utilization of Carbon Substrates

182 strains were examined for their ability to oxidize carbon sources using Biolog's automated identification system. All of the strains used in this study were subcultured onto BLA (Biolog Lactic Acid bacteria agar, Biolog, Inc. Hayward, CA, U.S.A. #70004) agar and incubated at 30°C for 48 h. Cells were scraped and suspended in BLA broth containing 0.56% (w/v) BLA

Table 1. Source of test strains used for analysis of metabolic profiles using Biolog.

Cluster	Strain	Source
M	*Leuconostoc amelibiosum* KCTC[a] 3524[T]	ATCC[b] 13146
	S33, S55, S63, S102, S128, S166, S168, S171, S177, S186, S5036, S5037, S5042, S5047, S5048, S5050, S5051, S5055, S5062, S5064, S5115, S5118, S5119, S5141, S5143, S5147, S5149, S5150, S5151, S5157, S5158, S5159, S5160, S5162, S5163, S5164, S5168, S5169, S5174, S5180, S5186, S5188, S5224, S5234, S5236, S5247, S5248, S5251, S5264, S5265, S5276, S5277, S5278	Korean fermented food (Kimchi)
N	*Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3505[T]	NCDO[c] 523; fermenting olives (8)
	S2, S140, S163, S172, S175, S239, S240, S247, S248, S255, S257, S259, S262, S263, S269, S272, S275, S277, S5178, S5184, S5216, S5225, S5226, S5285, S5286, S5287, S5290, S5297, S5300, S5302, S5303, S5379, S5382, S5385, S5388, S5390, S5394, S5395, S5398, S5400, S5406, S5412, S5413, S5414, S5417, S5422, S5473, S5474, S5479, S5482, S5488, S5490, S5493, S5497, S5499, S5502, S5507	Korean fermented food (Kimichi)
O	*Leuconostoc pseudomesenteroides* KCTC 3532[T]	ATCC 12291
	Leuconostoc lactis KCTC 3528[T]	ATCC 19256
	Lactobacillus animalis KCTC 3501[T]	NCDO 2425; dental plaque of baboon (4)
	Lactobacillus brevis KCTC 3498[T]	NCDO 1749; human feces (13)
	Lactobacillus parabuchneri KCTC 3503[T]	NCDO 2748; human saliva (6)
	S22, S92, S113, S170, S174, S187, S188, S197, S198, S207, S212, S217, S231, S232, S236, S5103, S5104, S5166, S5280, S5420	Korean fermented foo (Kimchi)
P	S5056, S5112, S5126, S5128, S5140, S5152, S5176, S5181, S5250, S5272, S5275	Korean fermented food (Kimchi)
Q	*Lactobacillus plantarum* KCTC 3103	NCDO 340
	Lactobacillus plantarum KCTC 3107	NCDO 1193
	Lactobacillus plantarum KCTC 3108[T]	NCDO 1752; pickled cabbage (13)
	Lactobacillus plantarum KCTC 3104	ATCC 10241; sauerkraut (13)
	Lactobacillus delbrueckii subsp. *delbrueckii* KCTC 1047[T]	IFO[d] 3202; distillery sour grain mash incubated at 45°C (13)
	Lactobacillus casei KCTC 3109[T]	NCDO 161; cheese (13)
	Lactobacillus plantarum KCTC 1048	ATCC 8014
	S73, S118, S192, S193, S194, S200, S210, S220, S222, S224, S225, S230, S234	Korean fermented food (Kimchi)
R	S1, S5041	Korean fermented food (Kimchi)
Single-1 (S-1)	*Leuconostoc citreum* KCTC 3526[T]	ATCC 49370; honeydew of rye ear (6)
Single-2 (S-2)	S3	Korean fermented food (Kimchi)
Single-3 (S-3)	S123-2	
Single-4 (S-4)	S136	
Single-5 (S-5)	S176	
Single-6 (S-6)	S178	
Single-7 (S-7)	S185	
Single-8 (S-8)	S199	
Single-9 (S-9)	S5299	
Single-10 (S-10)	S5386	
Single-11 (S-11)	S5393	
Single-12 (S-12)	S5486	

[a]KCTC: Korean Collection for Type Cultures, KRIBB, KIST, Taejon, Korea. [b]ATCC: American Type Culture Collection, Rockville, Md., U.S.A. [c]NCDO: NCFB, National Collection of Food Bacteria, Reading, UK. [d]IFO: Institute for Fermentation, Yodogawa-ku, Osaka, Japan. [T]Type strain.

broth (Biolog, Inc. Hayward, CA, U.S.A. #70014) and 0.016% (v/v) Tween 80 (Sigma, P-1754). The procedure followed was as indicated in the manual of the Biolog system (Biolog, Inc. Hayward, CA, U.S.A.). The optical density of each inoculum suspension was determined at 590 nm and adjusted to the appropriate level of approximately 35% to 42%. The Biolog GP microplates were preincubated at 30°C±5°C and inoculated with 150 μl of suspension per reaction well and then incubated at 30°C±5°C. In accordance with the manufacturer's instructions, the plates were placed into the plate reader (Biolog, Inc. Hayward, CA, U.S.A.) after incubation for 4 h and 24 h. The reader automatically measures the redox reaction colorimetrically and the well pattern is analyzed using Microlog release 3.50 software (Biolog, Inc. Hayward, CA, U.S.A.; 1, 10, 16, 17). Data obtained from the analysis of well patterns was used to compare and cluster the test strains using S_{SM} (simple matching; 14) and the UPGMA (unweighted pair group method with arithmetic averages; 14) using the computer program, CLUSTAN (18).

RESULTS AND DISCUSSION

The test strains were recovered in five major, one minor and twelve single clusters defined at the S_{SM} level of 80% (Fig. 1). These aggregate taxa were equivalent to the genus *Lactobacillus* (aggregate group Q and R), the genus *Leuconostoc* (aggregate group M and N) and the genera *Lactobacillus* and *Leuconostoc* (aggregate group O and P). The distribution of positive characters for each cluster are given in Table 2.

Cluster M consisted of 54 strains including *Leuconostoc amelibiosum* KCTC 3524 and cluster N contained 58 strains including *Leuconostoc mesenteroides* subsp. *mesenteroides* KCTC 3505. The strains belonging to the cluster M and N had the ability to oxidize D-fructose, D-gluconic acid, α-D-glucose, maltose and D-mannose. However, none of strains in cluster M and N had the ability to oxidize D-arabitol, L-fucose, D-galacturonic acid, α-keto valenic acid, L-malic acid, alaninamide, D-alanine, L-alanyl-glycine, 2'-deoxy adenosine, thymidine, thymidine-5'-monophosphate, glucose-6-phosphate or D-L-α-glycerol phosphate. The utilization of carbon sources such as D-psicose, D-xylose, pyruvic acid, lactulose and D-melibiose showed different patterns between cluster M and N.

Cluster O consisted of 25 strains including *Leuconostoc lactis* KCTC 3528, *Leuconostoc pseudomesenteroides* KCTC 3532, *Lactobacillus parabuchneri* KCTC 3503, *Lactobacillus brevis* KCTC 3498 and *Lactobacillus animalis* KCTC 3501. None of strains in cluster O had the ability to oxidize α-cyclodextrin, β-cyclodextrin, dextrin, glycogen, inulin, mannan, tween 80,

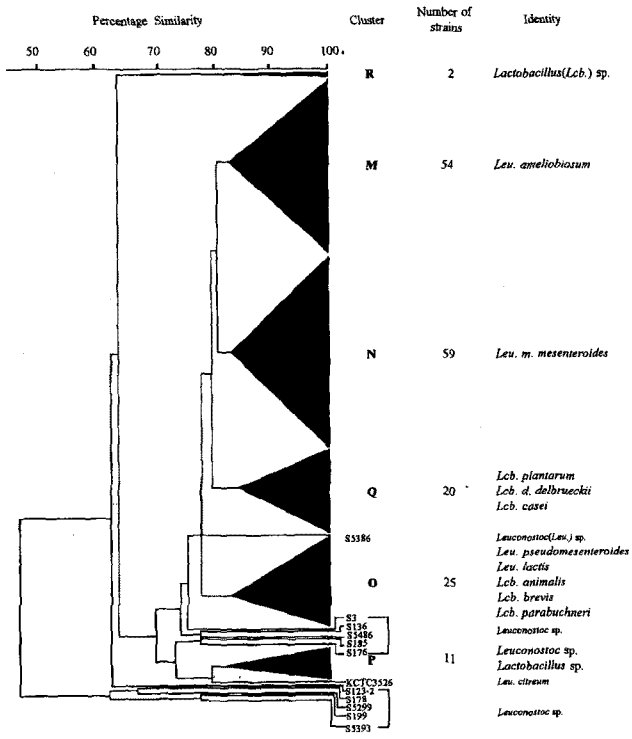

Fig. 1. Abridged dendrogram showing the relationships between the test strains defined in the S_{SM}, UPGMA analysis.

N-acetyl-D-mannosamine, amygdalin, D-arabitol, cellobiose, L-fucose, m-inositol, 3-methyl glucose, α-methyl D-glucoside, β-methyl D-glucoside, α-methyl D-mannoside, L-rhamnose, sedoheptulosan, D-tagatose, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, lactamide, D-malic acid, L-malic acid, mono-methyl succinate, propionic acid, alaninamide, D-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, 2, 3,-butanediol, 2'-deoxy adenosine, fructose-6-phosphate or glucose-6-phosphate. Cluster P contained only 11 isolates derived from kimchi. All strains belonging to cluster P had the ability to oxidize β-cyclodextrin, dextrin, N-acetyl-D-glucosamine, D-fructose, D-galactose, D-gluconic acid, α-D-glucose, maltose, D-mannose, D-psicose, D-ribose, salicin, sucrose, D-xylose, α-hydroxybutyric acid, β-hydroxybutyric acid, D-lactic acid methyl ester, methyl pyruvate, mono-methyl succinate and pyruvic acid. The very different patterns between cluster O and P are shown in Table 2.

Cluster Q consisted of the genus *Lactobacillus*, namely *Lactobacillus plantarum* KCTC 1048, *Lactobacillus plantarum* KCTC 3103, *Lactobacillus plantarum* KCTC 3104, *Lactobacillus plantarum* KCTC 3107, *Lactobacillus plantarum* KCTC 3108, *Lactobacillus delbrueckii* subsp. *delbrueckii* KCTC 1047 and *Lacto-*

Table 2. Average metabolic activities of test strains on 95 carbon sources in the Biolog GP microplate assay.

Carbon substrate		% of strains giving positive reactions																
Desig-nation	Name	M	N	O	P	Q	R	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12
A02	α-Cyclodextrin	4	10	0	55	0	0	0	0	0	0	100	100	0	100	100	100	100	0
A03	β-Cyclodextrin	13	5	0	100	0	0	0	0	100	0	100	100	0	100	100	0	100	0
A04	dextrin	69	10	0	100	30	50	100	0	100	100	100	100	100	100	100	100	100	0
A05	Glycogen	2	0	0	0	0	0	0	0	0	100	0	100	0	100	0	0	100	0
A06	Inulin	17	34	0	64	35	0	0	0	100	100	100	100	100	100	100	0	100	100
A07	Mannan	0	2	0	0	0	0	0	0	100	0	0	100	0	100	0	0	100	0
A08	Tween 40	7	9	12	18	5	0	0	0	0	0	0	100	0	100	0	100	0	0
A09	Tween 80	4	3	0	0	5	0	0	0	0	0	100	100	100	0	100	0	0	0
A10	N-Acetyl-D-glucosamine	98	100	56	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100
A11	N-Acetyl-D-mannosamine	4	0	0	9	35	0	0	100	0	100	0	100	0	0	100	0	0	0
A12	Amygdalin	26	17	0	55	74	0	100	0	0	0	100	100	100	100	0	0	0	0
B01	L-Arabinose	98	75	36	91	40	0	100	100	100	100	0	0	100	100	100	100	100	100
B02	D-Arabitol	0	0	0	0	0	50	0	0	100	0	0	100	0	100	100	0	100	0
B03	Arbutin	82	48	12	91	90	0	100	0	100	0	100	100	100	100	100	100	100	0
B04	Cellobiose	35	29	0	73	90	0	100	100	0	100	100	100	100	100	0	100	100	0
B05	D-Fructose	100	100	40	100	95	0	100	100	100	100	100	100	100	100	100	100	100	100
B06	L-Fucose	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	100	0
B07	D-Galactose	98	98	56	100	85	0	100	100	100	100	100	100	100	100	100	100	100	100
B08	D-Galacturonic acid	0	0	0	9	0	0	100	100	0	100	0	100	0	100	0	0	100	0
B09	Gentiobiose	28	27	4	82	80	0	100	100	0	0	100	100	100	100	100	0	0	0
B10	D-Gluconic acid	100	100	48	100	15	0	100	100	100	100	100	100	100	100	100	0	100	100
B11	α-D-Glucose	100	100	84	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100
B12	m-Inositol	4	0	0	0	0	0	0	0	0	0	0	100	100	100	0	0	100	0
C01	α-D-Lactose	15	10	8	0	25	0	0	0	0	0	0	100	0	100	100	0	0	0
C02	Lactulose	4	78	12	9	55	0	0	0	0	0	100	100	100	100	100	100	100	100
C03	Maltose	100	100	76	100	90	0	100	100	100	100	100	100	100	100	100	100	100	100
C04	Maltotriose	65	9	12	91	50	50	100	0	100	100	100	100	100	100	0	0	100	0
C05	D-Mannitol	67	56	20	82	100	0	100	100	100	100	0	100	100	100	100	0	100	100
C06	D-Mannose	100	100	36	100	95	0	100	100	0	100	100	100	100	100	100	100	100	100
C07	D-Melezitose	4	0	8	9	85	50	0	0	0	0	0	100	0	100	100	0	100	0
C08	D-Melibiose	9	95	56	0	20	0	0	100	100	100	100	100	0	100	100	100	100	100
C09	α-Methyl D-galactoside	7	58	16	9	0	0	0	0	0	100	100	100	0	100	100	100	100	100
C10	β-Methyl D-galactoside	46	66	28	46	60	0	100	0	100	100	100	100	100	100	100	100	100	100
C11	3-Methyl glucose	4	5	0	0	5	50	0	0	0	0	0	100	0	100	100	0	100	0
C12	α-Methyl D-glucoside	2	32	0	0	0	0	100	100	0	0	100	100	100	100	100	0	100	0
D01	β-Methyl-D-glucoside	63	24	0	73	90	50	100	0	0	0	100	0	100	100	100	100	0	0
D02	α-Methyl-D-mannoside	2	10	0	9	0	0	0	0	0	0	0	100	100	100	0	100	100	0
D03	Platinose	7	19	4	0	0	0	100	100	0	0	100	100	100	100	0	0	100	0
D04	D-Psicose	100	31	8	100	15	0	100	100	100	100	100	100	100	100	100	0	100	100
D05	D-Raffinose	2	63	24	18	5	50	0	0	0	100	100	100	0	100	100	0	100	100
D06	L-Rhamnose	6	0	0	36	5	50	100	0	0	100	0	100	100	100	0	0	100	0
D07	D-Ribose	98	81	28	100	65	0	100	100	100	100	100	100	100	100	100	100	100	100
D08	Salicin	80	93	4	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D09	Sedoheptulosan	2	0	0	9	0	0	100	0	0	0	0	100	0	100	100	100	0	100
D10	D-Sorbitol	0	5	8	0	60	0	0	0	0	0	0	0	100	100	0	0	0	100
D11	Stachyose	0	9	20	0	0	0	0	0	0	0	100	100	100	100	0	0	0	0
D12	Sucrose	98	97	36	100	80	100	100	100	0	100	100	100	100	100	100	100	100	100

Table 2. Continued.

Carbon substrate		% of strains giving positive reactions																
Desig-nation	Name	M	N	O	P	Q	R	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12
E01	D-Tagatose	15	3	0	64	15	50	100	100	0	0	0	0	0	100	0	100	0	0
E02	D-Trehalose	4	97	8	9	90	0	100	100	0	0	100	100	100	100	100	100	100	100
E03	Turanose	4	15	8	9	40	0	100	100	100	0	100	100	100	100	100	0	100	0
E04	Xylitol	2	0	4	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0
E05	D-Xylose	100	54	8	100	0	50	100	100	100	100	100	0	0	100	100	100	100	100
E06	Acetic acid	4	14	4	46	0	100	100	0	0	100	100	100	100	100	100	0	100	100
E07	α-Hydroxybutyric acid	19	9	0	100	0	0	100	0	100	100	100	100	100	100	100	0	100	100
E08	β-Hydroxybutyric acid	28	14	0	100	0	0	100	0	100	100	100	100	100	100	100	0	100	0
E09	γ-Hydroxybutyric acid	13	10	0	64	0	100	100	0	0	0	0	100	0	100	0	100	100	100
E10	ρ-Hydroxyphenyl acetic acid	7	9	4	82	0	50	100	0	100	0	100	0	0	100	0	0	100	0
E11	α-Keto glutamic acid	2	3	8	9	5	0	100	0	0	0	100	0	0	100	100	0	100	0
E12	α-Keto valenic acid	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F01	Lactamide	6	5	0	82	0	0	100	0	0	0	0	100	0	100	0	0	0	0
F02	D-Lactic acid methyl ester	63	7	4	100	20	0	100	0	100	0	100	100	100	100	100	0	100	100
F03	L-Lactic acid	32	3	8	73	0	0	0	0	100	0	0	100	0	100	100	0	100	100
F04	D-Malic acid	0	2	0	0	0	0	0	0	100	0	0	0	0	100	100	0	100	100
F05	L-Malic acid	0	0	0	0	10	0	0	0	0	0	0	0	0	100	0	0	0	0
F06	Methyl pyruvate	74	46	20	100	10	0	0	0	100	0	100	0	0	100	100	100	100	100
F07	mono-methyl succinate	7	2	0	100	0	50	100	100	0	100	100	0	0	100	0	0	100	100
F08	Propionic acid	11	22	0	91	0	50	100	100	0	100	100	100	100	100	100	100	100	0
F09	Pyruvic acid	89	2	4	100	0	100	100	0	0	100	100	0	0	100	100	0	100	100
F10	Succinamic acid	2	3	12	36	5	100	100	0	0	0	0	0	0	100	0	0	100	0
F11	Succinic acid	0	3	4	0	5	0	0	0	0	0	0	0	0	100	0	0	100	0
F12	N-Acetyl L-glutamic acid	0	3	4	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0
G01	Alaninamide	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0
G02	D-Alanine	0	0	0	9	0	100	0	0	0	100	0	100	0	100	0	0	0	0
G03	L-Alanine	0	2	4	0	5	50	0	0	100	100	0	0	0	100	100	0	100	0
G04	L-Alanyl-glycine	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	0	100	0
G05	L-Asparagine	2	0	0	0	0	0	0	0	100	0	0	0	0	100	0	0	100	0
G06	L-Glutamic acid	0	2	0	9	0	100	0	0	100	0	0	0	0	100	100	0	100	0
G07	Glycyl-L-glutamic acid	0	3	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0
G08	L-Pyroglutamic acid	2	3	0	9	5	100	0	0	0	0	0	0	0	100	0	0	0	0
G09	L-Serine	4	2	0	0	0	0	0	0	0	0	0	0	0	100	100	0	100	0
G10	Putrescine	2	5	8	0	5	100	0	0	100	0	0	0	0	100	100	0	100	0
G11	2,3-Butanediol	13	17	0	82	0	0	100	0	0	0	0	100	100	100	0	0	100	0
G12	Glycerol	4	14	4	46	80	100	100	0	0	0	0	0	0	100	100	0	100	0
H01	Adenosine	2	9	40	18	0	0	0	0	100	0	0	0	0	0	0	0	0	0
H02	2'-Deoxy adenosine	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	100	0
H03	Inosine	2	14	36	0	5	50	0	0	100	0	0	0	0	100	100	0	100	0
H04	Thymidine	0	0	4	0	0	0	0	0	100	0	0	0	0	100	0	0	100	0
H05	Uridine	2	10	44	9	5	50	0	0	100	0	0	0	0	100	0	0	0	0
H06	Adenosine-5'-monophosphate	0	2	20	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0
H07	Thymidine-5'-monophosphate	0	0	4	0	0	0	0	0	100	0	0	0	0	100	100	100	0	0
H08	Uridine-5'-monophosphate	0	2	28	9	0	0	0	0	100	0	0	0	0	100	100	0	100	0
H09	Fructose-6-phosphate	11	0	0	91	0	100	100	100	0	100	100	100	0	100	100	0	0	0
H10	Glucose-1-phosphate	0	2	4	0	0	50	0	100	0	0	0	0	0	0	0	0	0	0
H11	Glucose-6-phosphate	0	0	0	0	0	0	0	100	100	0	0	0	0	100	0	0	0	0
H12	D-L-α-Glycerol phosphate	0	0	4	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0

bacillus casei KCTC 3109. All strains in cluster Q oxidized only a few carbon substrates, e.g. N-acetyl-D-glucosamine, α-D-glucose, D-mannitol and salicin. However, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, maltose, D-mannose, D-melezitose, β-methyl-D-glucoside, sucrose, D-trehalose, and glycerol were oxidized by more than 80% of strains belonging to cluster Q. Only two isolates from kimchi were assigned to cluster R and the strains were identified as belonging to the genus *Lactobacillus*, but they displayed a very low similarity level of less than 20%. Most carbon sources were not oxidized by the two strains in cluster R.

Twelve strains were assigned to single-membered clusters including *Leuconostoc citreum* KCTC 3526. All were identified as belonging to the genus *Leuconostoc*. Single-membered cluster 1, *Leuconostoc citreum* KCTC 3526, is closely related to cluster P (Fig. 1). Single-membered clusters 5 (s176) and 7 (s185) are also related to cluster P and single-member cluster 1 (Fig. 1). Also, single-membered clusters 4 (s136) and 12 (s5486) have a similarity level of 78% and are the next most closely related to single-membered cluster 2 (s3). Single-membered cluster 2 is distantly related to the major clusters. Single-membered cluster 10 (s5386) is distant from other single-membered clusters and from the related clusters M, N and Q. Single-membered clusters 3 (s123-2), 6 (s178), 8 (s199), 9 (s5299) and 11 (s5393) presented various carbon-source utilization patterns and formed a very different grouping pattern to that of the other isolates derived from kimchi (Fig. 1).

Many studies have used the Biolog system. Vauterin *et al.* (17) reported the classification of *Xanthomonas* using Biolog. Garland *et al.* (7) studied the classification and characterization of heterotrophic microbial communities using this system. These reports indicate that the Biolog system is useful in the classification and characterization of bacteria. It offers a rapid, standardized and computerized approach to bacterial characterization.

The genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* are known to play an important role in the fermentation of kimchi (2, 3, 11, 12). But it is currently difficult to distinguish these strains due to the their ambiguous taxonomic status. This study attempted to determine the taxonomic status of these genera and to aid their rapid classification and identification using the Biolog system. In the future, using the test data obtained from Biolog we hope to be able to construct a customized database for the identification of isolates derived from kimchi.

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