The Journal of Microbiology, March 1997, p.10-14

Vol. 35, No. 1

Identification of *Leuconostoc* Strains Isolated from Kimchi Using Carbon-source Utilization Patterns

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(Received December 21, 1996 / Accepted February 10, 1997)

The database of metabolic fingerprints generated using the Biolog system of lactic acid bacteria isolated from kimchi, described by Lee *et al.* (8), was used for the identification of 75 *Leuconostoc* isolates. The test strains were isolated using a selective isolation medium specific for the genus *Leuconostoc* and examined for their ability to oxidize carbon sources using the Biolog system. The results show that the 75 test strains were identified to the known *Leuconostoce* clusters. It is suggested that the Biolog system can be applied for rapid identification of lactic acid bacteria isolated from kimchi.

Key words: Biolog GP microplate assay, Lactic acid bacteria

Kimchi is a fermented vegetable food found in Korea. The flavor of kimchi is dependent upon the ingredients, temperature, and the bacteria involved in the fermentation process (2, 7, 9). In particular, the genera *Lactobacillus, Leuconostoc* and *Pediococcus* are known to play an important role in kimchi fermentations (2, 7, 9). The genera Lactobacillus, *Leuconostoc* and *Pediococcus* have similar physiological and biochemical characteristics (4). Phylogenetically, these three genera are considered intermixed (11, 14). Therefore, there is a strong 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 and is based on the test strains ability to oxidize 95 different carbon substrates which include amino acids, carboxylic acids and carbohydrates. The resultant metabolic fingerprints of the test strains are compared with those of strains in a database (1, 3, 5, 12, 13).

In a previous paper (8), a database of metabolic fingerprints, through use of the Biolog system (Biolog, Inc., Hayward, CA, U.S.A.) was generated by the author. The database comprised data derived from 182 lactic acid bacteria isolated from kimchi, including 15 reference strains of lactic acid bacteria. The database comprised 5 major, 1 minor and 12 single-membered clusters, based on the S_{SM} (simple matching; 10) and UPGMA (unweighted pair group method with arithmetic averages; 10) algorithms, at a 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.

In this study, we used this database for the rapid identification of 75 presumptive *Leuconostoc* strains isolated from kimchi.

Materials and Methods

Strains

All of the test strains were isolated from kimchi using a *Leuconostoc* selective isolation medium, i.e. phenylethyl alcohol sucrose (PES) medium. The composition of the medium was; trypticase peptone 5g, 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 (6). Seventy five presumptive *Leuconostoc* strains were isolated and are listed in Table 1.

Utilization of carbon substrates

The presumptive *Leuconostoc* strains were ex-

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Table 1. 75 *Leuconostoc* isolates identified to known clusters B, C and D

Cluster	Strain(Lu)
M	19, 227
N	4, 5, 9, 14, 28, 31, 32, 38, 42, 50, 55, 56, 61, 62, 87, 88, 92, 94, 95, 100, 102, 109, 117, 124, 129, 130, 134, 148, 153, 154, 155, 161, 161-1, 167, 177, 186, 187, 188, 192, 196, 205, 210, 251, 252, 253, 267, 275, 290
O	13, 15, 91, 97, 103, 104, 108, 110, 112, 114, 133-1, 141, 149, 157, 165, 200
P	10, 29
Single-13(S-13)	7
Single-14(S-14)	53
Single-15(S-15)	63
Single-16(S-16)	68
Single-17(S-17)	137
Single-18(S-18)	284
Single-19(S-19)	559

amined for their ability to oxidize different carbon sources using Biolog's automated identification system. The test strains were subcultured onto BLA (Biolog Lactic Acid bacteria agar, Biolog, Inc. Hayward, CA, U.S.A. #70004) agar and incubated at 30°C for 48h. The procedure followed was as prescribed in the manual of the Biolog system (Biolog, Inc. Hayward, CA, U.S.A.) as described in a previous paper (8). The utilization patterns of carbon sources were analyzed using Microlog release 3.50 software (Biolog, Inc. Hayward, CA, U.S.A.; 1, 5, 12, 13). The identification of the test strains was achieved by comparison with the database described previously (8).

Results and Discussion

Test strains were identified by comparison with the database described by Lee *et al.* (8). All 75 strains were identified to known clusters at the S_{sm} level of 80%. The distribution of positive characters for each cluster are given in Table 2. None of the strains had the ability to oxidize inulin, mannan, N-acetyl L-glutamic acid, alaninamide, D-alanine, L-alanine, L-asparagine, glycyl-L-glutamic acid, 2′-deoxyadenosine, inosine, thymidine, uridine, adenosine-5′-monophosphate and thymidine-5′-monophosphate.

Fifty strains were found to belong to cluster M and N, equivalent to the genus *Leuconostoc*. Two isolates, Lu-19 and Lu-227, were found to belonging to cluster M. Forty eight isolates, listed in Table 1, were characterized as belonging to cluster N. The strains belong to clusters M and N had the ability to oxidize N-acetyl-D-glucosamine, D-fructose, α-D-glucose, maltose and D-mannose. However, none of the strains in cluster M and N had the ability to oxidize glycogen, N-acetyl-D-mannosamine, m-inositol, α-methyl-D-mannoside, α-keto glutamic acid, α-keto valenic acid, lactamide, methyl pyruvate, succinic acid, glycerol and glucose-6-phosphate. Only Lu-31 in cluster N was able to utilize L-malic acid, L-alanylglycine and L-glutamic acid. L-pyroglutamic acid and uridine-5′-monophosphate were oxidized only by Lu-134 in cluster N.

Eighteen isolates, which belonged to clusters O and P, were identified to the genus *Leuconostoc* by the Biolog system. Sixteen of the 18 isolates belonged to cluster O and the remaining were assigned to cluster P. None of the strains in clusters O and P had the ability to oxidize glycogen, inulin, mannan, L-fucose, α-methyl D-glucoside, palatinose, D-raffinose, sedoheptulosan, D-trehalose, α-keto valenic acid, L-malic acid, N-acetyl L-glutamic acid, alaninamide, D-alanine, l-alanine, L-alanyl-glycine, L-asparagine, L-glutamic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, adenosine, 2′-deoxy adenosine, inosine, thymidine, uridine, adenosine-5′-monophosphate, thymidine-5′-monophosphate, uridine-5′-monophosphate, fructose-6-phosphate, glucose-1-phosphate and D-L-α-glycerol phosphate. The differing substrate utilization patterns between cluster O and P are shown in Table 2. In a previous study also using the Biolog system (8), the genera *Lactobacillus* and *Leuconostoc* were found to be mixed in together clusters O and P. Therefore, it was difficult for these isolates to be identified to either *Leuconostoc* or *Lactobacillus*, despite the eighteen strains being isolated using a *Leuconostoc* selective medium and being identified to the genus *Leuconostoc* by the Biolog system. More detailed study is required to allow clear distinctions to be made regarding the taxonomic status of isolates assigned to these clusters.

Seven isolates were assigned to single-membered clusters. All were identified as belonging to the genus *Leuconostoc* and of being closely related to those single-membered clusters described previously (8). Single-membered cluster 13 (Lu-7) is related to single-membered cluster 7 (s185) described in a previous paper (8) and single-member clusters 14 (Lu-53), 15 (Lu-63) and 16 (Lu-68) are related to single-membered cluster 12 (s5486) described previously (8). Single-member clusters 17 (Lu-13) and 18 (Lu-284) are related to single-membered cluster 19 (Lu-559). Single-membered cluster 19 (Lu-559) is related to single-membered cluster 1, *Leuconostoc citreum* KCTC 3526, which is closely related to cluster P.

Table 2. Average metabolic activities of *Leuconostoc* isolates on 95 carbon sources in the Biology GP microplate assay

Carbon substrate		% of strains giving positive reactions										
Desig-nation	Name	M	N	O	P	S-13	S-14	S-15	S-16	S-17	S-18	S-19
A02	α-Cyclodextrin	50	15	31	100	0	0	100	0	0	0	0
A03	β-Cyclodextrin	0	2	0	50	0	0	0	0	0	0	100
A04	Dextrin	50	4	0	100	0	0	0	0	100	100	100
A05	Glycogen	0	0	0	0	0	0	0	0	0	0	100
A06	Inulin	0	0	0	0	0	0	0	0	0	0	0
A07	Mannan	0	0	0	0	0	0	0	0	0	0	0
A08	Tween 40	0	4	6	0	0	0	0	0	0	0	0
A09	Tween 80	0	4	6	0	0	0	0	0	0	0	100
A10	N-Acetyl-D-glucosamine	100	100	13	100	100	100	100	100	100	100	100
A11	N-Acetyl-D-mannosamine	0	0	6	0	100	0	0	100	0	0	0
A12	Amygdalin	50	31	6	100	0	0	0	100	0	0	0
B01	L-Arabinose	50	92	25	100	0	100	100	100	100	100	100
B02	D-Arabitol	0	2	13	0	0	0	100	0	0	0	0
B03	Arbutin	50	46	6	100	0	100	0	0	0	100	100
B04	Cellobiose	50	73	0	100	0	100	0	100	0	0	100
B05	D-Fructose	100	100	13	100	0	100	100	100	100	100	100
B06	L-Fucose	0	4	0	0	0	0	0	0	100	100	0
B07	D-Galactose	50	100	13	100	0	100	0	100	100	100	100
B08	D-Galacturonic acid	0	2	0	50	0	0	100	0	0	100	0
B09	Gentiobiose	50	77	6	100	100	0	100	100	100	100	100
B10	D-Gluconic acid	100	94	19	100	0	0	0	100	0	0	100
B11	α-D-Glucose	100	100	25	100	100	100	100	100	100	100	100
B12	m-Inositol	0	0	6	0	0	0	0	0	0	0	0
C01	α-D-Lactose	50	2	6	0	0	0	100	0	100	0	100
C02	Lactulose	0	90	13	0	0	0	0	100	100	100	0
C03	Maltose	100	100	19	100	100	100	100	100	100	100	100
C04	Maltotriose	0	6	13	50	0	0	0	0	100	100	100
C05	D-Mannitol	0	69	0	50	0	0	0	0	0	100	0
C06	D-Mannose	100	100	13	100	100	100	100	100	100	100	100
C07	D-Melezitose	0	6	19	0	0	0	0	0	0	0	0
C08	D-Melibiose	0	100	19	50	100	100	0	100	100	100	0
C09	α-Methyl D-galactoside	0	60	13	0	100	100	100	0	0	100	0
C10	β-Methyl D-galactoside	0	65	13	100	100	100	0	100	100	100	0
C11	3-Methyl glucose	0	4	13	0	100	0	0	0	0	100	0
C12	α-Methyl D-glucoside	0	42	0	0	0	0	0	100	0	100	0
D01	β-Methyl-D-glucoside	100	31	0	100	0	0	0	0	0	0	0
D02	α-Methyl-D-mannoside	0	0	13	0	0	0	100	100	0	100	0
D03	Palatinose	0	52	0	0	0	0	100	0	100	100	0
D04	D-Psicose	100	40	19	100	0	0	0	0	100	100	100
D05	D-Raffinose	0	71	0	0	100	100	0	0	100	100	0
D06	L-Raffinose	0	21	6	50	0	0	0	0	100	100	100
D07	L-Rhamnose	100	96	63	100	0	100	100	100	100	100	0
D08	D-Ribose	100	94	6	100	0	100	100	100	100	100	100
D09	Salicin	0	17	0	0	100	100	0	100	0	100	0
D10	Sedoheptulosan	0	6	13	0	0	100	0	0	0	100	0
D11	Stachyose	0	13	6	50	100	0	0	0	100	100	0
D12	Sucrose	50	98	13	100	0	100	100	100	100	100	100
E01	D-Tagatose	50	23	6	50	0	0	0	0	100	100	100
E02	D-Trehalose	0	98	0	0	0	0	100	100	100	100	100
E03	Turanose	0	27	6	0	0	0	0	0	0	100	0
E04	Xylitol	0	2	6	0	0	0	0	0	0	0	0
E05	D-Xylose	100	75	31	100	0	100	0	0	100	100	100
E06	Acetic acid	0	8	0	50	0	0	0	0	100	100	0
E07	α-Hydroxybutyric acid	0	4	0	100	0	0	0	0	100	100	100
E08	β-Hydroxybutyric acid	0	10	0	50	0	0	0	0	100	100	100
E09	γ-Hydroxybutyrie acid	0	8	13	100	0	0	0	0	100	0	0

Table 2. Countinued

Carbon substrate		% of strains giving positive reactions										
Designation	Name	M	N	O	P	S-13	S-14	S-15	S-16	S-17	S-18	S-19
E10	ρ-Hydroxyphenyl acetic acid	0	6	13	50	100	100	0	100	0	100	100
E11	α-Keto glutamic acid	0	0	6	50	100	0	100	0	100	0	100
E12	α-Keto valenic acid	0	0	0	0	0	0	0	0	0	0	00
F01	Lactamide	0	0	0	100	0	0	0	0	100	0	0
F02	D-Lactic acid methyl ester	50	10	0	100	0	0	0	0	0	100	100
F03	L-Lactic acid	0	6	13	50	0	0	0	0	0	0	100
F04	D-Malic acid	0	2	6	0	0	0	0	0	0	0	0
F05	L-Malic acid	0	2	0	0	0	0	0	0	0	0	0
F06	Methyl pyruvate	0	0	0	50	0	0	0	0	0	0	100
F07	mono-methyl succinate	0	4	0	100	0	0	0	0	100	0	100
F08	Propionic acid	0	8	13	100	0	0	100	0	100	100	0
F09	Pyruvic acid	0	10	13	50	100	0	0	0	100	0	100
F10	Succinamic acid	0	2	6	0	100	100	0	100	0	0	100
F11	Succinic acid	0	0	6	0	100	0	0	100	0	100	0
F12	N-Acetyl L-glutamic acid	0	0	0	0	0	0	0	0	0	0	0
G01	Alaninamide	0	0	0	0	0	0	0	0	0	0	0
G02	D-Alanine	0	0	0	0	0	0	0	0	0	0	0
G03	L-Alanine	0	0	0	0	0	0	0	0	0	0	0
G04	L-Alanyl-glycine	0	2	0	0	0	0	0	0	0	0	0
G05	L-Asparagine	0	0	0	0	0	0	0	0	0	0	0
G06	L-Glutamic acid	0	2	0	0	0	0	0	0	0	0	0
G07	Glycyl-L-glutamic acid	0	0	0	0	0	0	0	0	0	0	0
G08	L-Pyroglutamic acid	0	2	0	0	0	0	0	0	0	0	0
G09	L-Serine	0	4	0	0	0	0	0	0	0	0	0
G10	Putrescine	0	4	0	0	0	100	0	100	0	100	100
G11	2,3-Butanediol	0	2	6	50	100	0	0	100	100	0	0
G12	Glycerol	0	0	6	100	0	0	0	0	100	100	0
H01	Adenosine	50	0	0	0	0	100	0	0	0	0	0
H02	2'-Deoxy adenosine	0	0	0	0	0	0	0	0	0	0	0
H03	Inosine	0	0	0	0	0	0	0	0	0	0	0
H04	Thymidine	0	0	0	0	0	0	0	0	0	0	0
H05	Uridine	0	0	0	0	0	0	0	0	0	0	0
H06	Adenosine-5'-monophosphate	0	0	0	0	0	0	0	0	0	0	0
H07	Thymidine-5'-monophosphate	0	0	0	0	0	0	0	0	0	0	0
H08	Uridine-5'-monophosphate	0	2	0	0	0	0	0	0	0	0	0
H09	Fructose-6-phosphate	0	10	0	0	0	0	0	0	0	0	0
H10	Glucose-phosphate	0	2	0	0	100	100	0	0	0	0	0
H11	Glucose-6-phosphate	0	0	6	0	0	0	0	100	0	0	0
H12	D-L-α-Glycerol phosphate	0	0	0	0	0	0	0	0	0	0	0

None of the isolates were found to belong to clusters Q or R, which are equivalent to the genus *Lactobacillus*. It is concluded that the carbon-source utilization patterns, generated using the Biolog system, can be used for the identification of presumptive *Leuconostoc* strains isolated from kimchi. The Biolog system is an automated identification and classification system for microorganisms. It is a much faster and less laborious identification technique than other classical identification tools. Furthermore, it has a comprehensive database to which test results may be compared. It is suggested that this method can be applied for the rapid identification of lactic acid bacteria.

Acknowledgments

This work was supported by grants from Korean Ministry of Agriculture and Forestry.

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