

Identification and Antigenotoxic Activity of *Leuconostoc* strains from Leek Kimchi

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I. Introduction

Lactic acid bacterium strains isolated from fermented leek kimchi showed antigenotoxic effects and antioxidative effects^{1),2),3)}. We isolated lactic acid bacteria from leek kimchi and identified phenotypic characterization. The genotypic characterization determined by Rep-PCR and RAPD-PCR. We select *Leuconostoc* strains and used the comet assay in order to investigate the antigenotoxic activity of them.

II. Method

The method of identified cell morphology gram-positive, catalase negative was described by Gerhardt et al.⁴⁾. Growth at 10°C, 45 °C, 6.5% (w/v) NaCl, pH 3.9 and 9.6 followed in the phenotypic differentiation were according to the information supplied by Wood et al.⁵⁾ and Stiles et al.⁶⁾. Lactic acid configuration was determined by commercial kit(Hoffman La Roche Diagnostic Mannheim) and meso-DAP test was determined according to a modified method of Schillinger et al.⁷⁾. The total genomic DNA from LAB was isolated according to the method described by Pitcher et al.⁹⁾. Rep-PCR was used for identification of lactic acid bacteria strains from Kimchi more accurately, using the primer GTG5 (5`-GTG GTG GTG GTG GTG-3`) and methods described by Gevers et al¹⁰⁾. RAPD-PCR was done using the primer M13 (5`-GAG GGT GGC GGT TCT-3`). DNA was amplified using methods and amplification conditions described by Andrighetto et al¹¹⁾. The comet assay was performed basically according to Singh et al.¹²⁾ with a slight modification.

III. Result

In total, 29 strains were isolated from leek kimchi and identified to genus level

by phenotypic characterization. 19 strains were found to belong to the genus *Leuconostoc*, 2 strains to *Lactobacillus plantarum*, 7 strains to homofermented *Lactobacillus*, 1 isolate to heterofermented *Lactobacillus* (Table 1).

Twelve strains (LK15, LK11*, LK4, LK2, LK10, LK23, LK1*, LK16, LK18, LK21, LK12 and LK5*) can be identified as *L. mesenteroides* ssp. *mesenteroides*. Five strains (LK7, LK9*, LK8, LK19*, LK3) could belong to either *Leuconostoc kimchi*, *Weissella kimchi*

Table 1. Phenotypic Identification

No.	Mor	Ga	Ca	Gr	10	45	3.9	9.6	6.5	La	Ar	m	ID
LK 1	coccoid, pairs	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 2	coccoid, pairs	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 3	coccoid, pairs	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 4	coccoid, pairs	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 5	coccoid, pairs	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 6	coccus, chain	+	-	+	+	-	w	-	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 7	rods, short, chain	+	-	+	+	-	+	+	+	D	+	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 8	rods, short, chain	+	-	+	+	-	+	+	+	D	+	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 9	rods, short, chain	+	-	+	+	-	+	+	+	D	+	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 10	rods, short, chain	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 11	coccus, chain, round	+	-	+	+	-	+	-	-	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 12	ovoid	+	-	+	+	-	+	-	-	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 13	short, rod, chain	-	-	+	+	-	+	-	-	L	-	-	<i>Lactobacillus</i> spp
LK 14	rod, chain	-	-	+	+	-	+	-	-	L	-	-	<i>Lactobacillus</i> spp
LK 15	coccoid, chain	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 16	rods, short, chain	+	-	+	+	-	+	+	+	D	+	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 17	rod, chain	-	-	+	+	-	-	-	-	L	-	-	<i>Lactobacillus</i> spp
LK 18	coccoid, chain	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 19	coccoid, chain	+	-	+	+	-	d	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 20	rods, short, chain	-	-	+	+	-	+	+	+	L	-	-	<i>Lactobacillus</i> spp
LK 21	coccoid, chain	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 22	rods, short, chain	+	-	+	+	-	+	+	+	DL	-	-	<i>Lactobacillus</i> spp
LK 23	coccoid, chain	+	-	+	+	-	+	+	+	D	-	-	<i>Leuconostoc</i> or <i>Weissella</i>
LK 24	short, rod, chain	-	-	+	+	-	+	-	-	L	-	-	<i>Lactobacillus</i> spp
LK 25	rod, long	-	-	+	+	-	+	-	w	L	-	-	<i>Lactobacillus</i> spp
LK 26	coccus, pairs	+	-	+	+	-	-	-	-	D	-	-	<i>Lactobacillus</i> spp
LK 27	short, rod, chain	-	-	+	+	-	+	-	-	L	-	-	<i>Lactobacillus</i> spp
LK 28	short rod chain, some pairs	-	-	+	+	+	+	-	-	DL	-	+	<i>Lactobacillus plantarum</i>
LK 29	short rod chain, some pairs	-	-	+	+	+	+	-	+	DL	-	+	<i>Lactobacillus plantarum</i>

Mor: Cell-morphology, Ga: Gas production, Ca: catalase activity, Gr: Gram characterization, 10: Growth at 10°C, 45: Growth at 45°C, 3.9: Growth at pH 3.9, 9.6: Growth at pH 9.6, 6.5: Growth at NaCl 6.5%, La: Lactic acid isomer, Ar: Arginine hydrolysis, m: meso-DAP, ID: Identification by Phenotypic characterization

or *Weissella thailandensis*. One strain(LK 26*) clustered together with the *L.gelidum* and *L.fructosum*. (Fig. 1). The results of RAPD-PCR typing are shown group I (LK16, LK23, LK4, LK18, LK2, LK10, LK15, LK1* and LK21) may be multiple isolates. (LK 5*). The strains LK 11* and LK 12 of group I may be the same strain. Group III(LK 24* and LK 6*) may indicate that the determination of its morphology may have been incorrect. Strains LK19* and LK3 of group IV(LK19*, LK9*, LK26*, LK3, LK8 and LK7) may be multiple isolated of the same strain. The strain LK 26* showed a completely different fingerprint than other cocci in RAPD-PCR and also in rep-PCR. (Fig. 1,2). Only one obligately heterofermentative rod(LK 22*) was isolated. This strain was

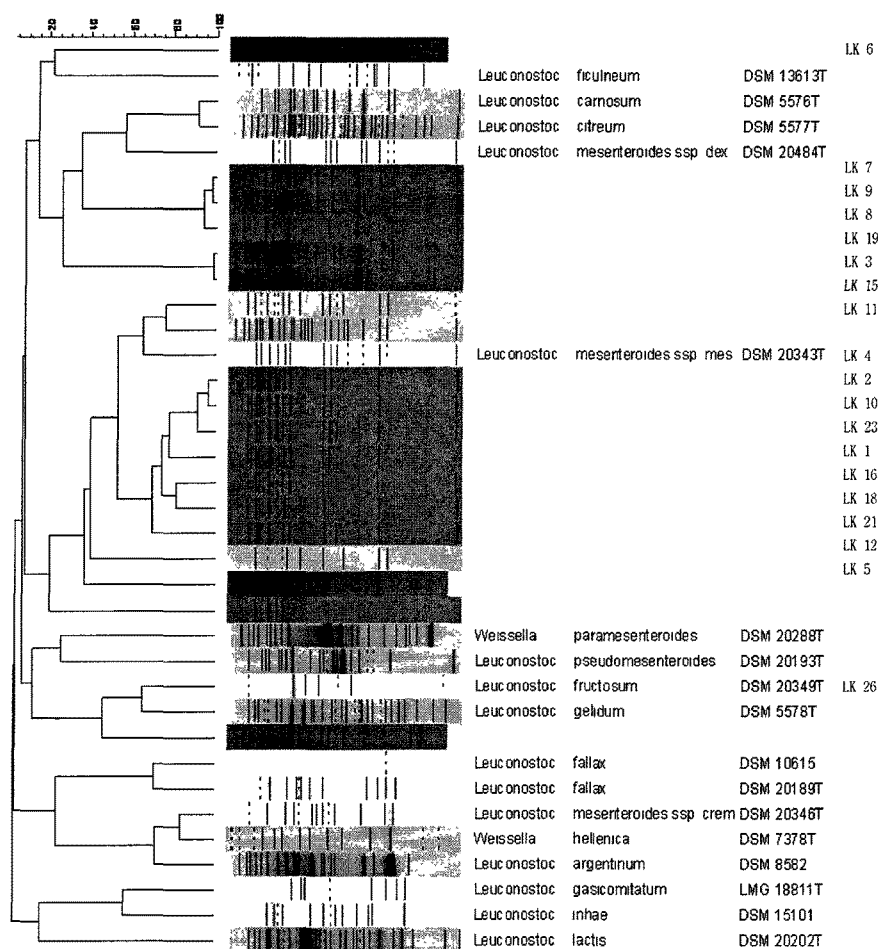


Fig. 1. Rep-PCR Fingerprints of *Leuconostec*, *Weissella* reference strains and Kimchi strains.

neither *W.confusa* nor *L.buchneri* but either a new species or *W.kimchi*. Two facultatively heterofermentative strains from Kimchi (LK 28 and 29) clustered together with the *L. plantarum* type strain in the RAPD-PCR analysis. (Figure. 3). The other obligately homofermentative rods (LK27, LK25, LK14 and LK17*)clustered closely together in the RAPD analysis in group II indicating that they probably belong to the same species. Twelve strains (LK11, LK1, LK26, LK5, LK6, LK9, LK19, LK22, LK17, LK24 ,LK13 and LK20) are recommended for sequencing. Isolated *Leuconostoc* strains was showing significant antigenotoxic effects except strains LK 6 and LK 26.(Table 3)

IV. Discussion

Biofunctional activity of leek kimchi may be increase antigenotoxic activity of *Leuconostoc* strains. They were main lactic acid bacteria of fermented kimchi. Selection of antigenotoxic *Leuconostoc* strains will be possible to make anticarcinogenic Kimchi and functional fermentated foods.

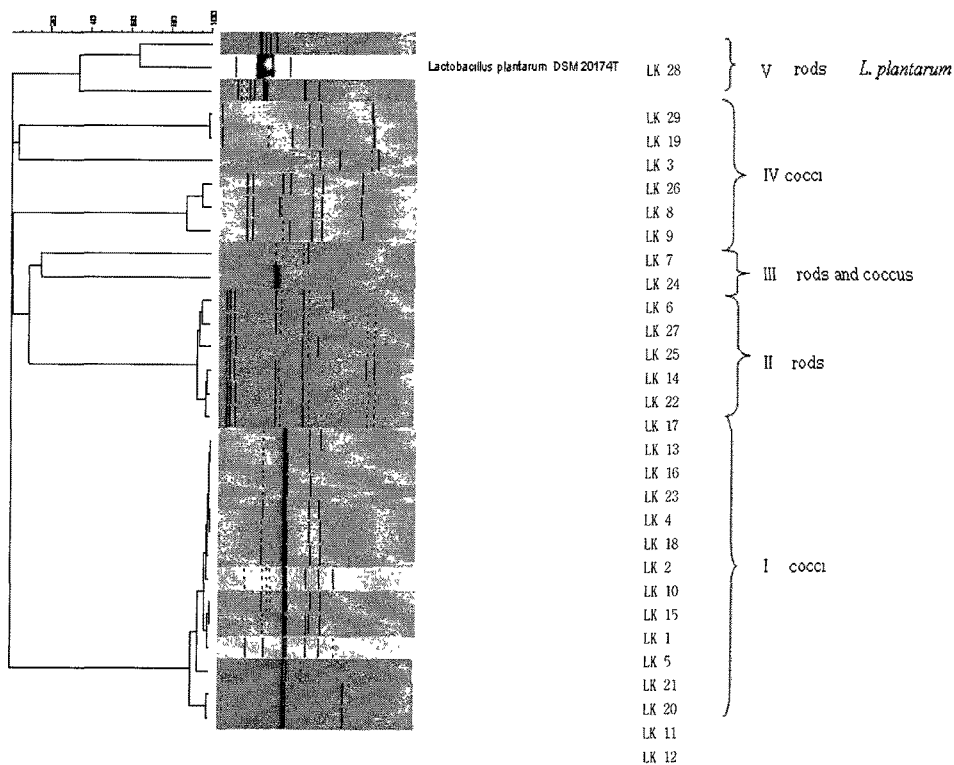


Fig. 2. RAPD-PCR fingerprint clustering of *L. plantarum* reference culture and Kimchi.

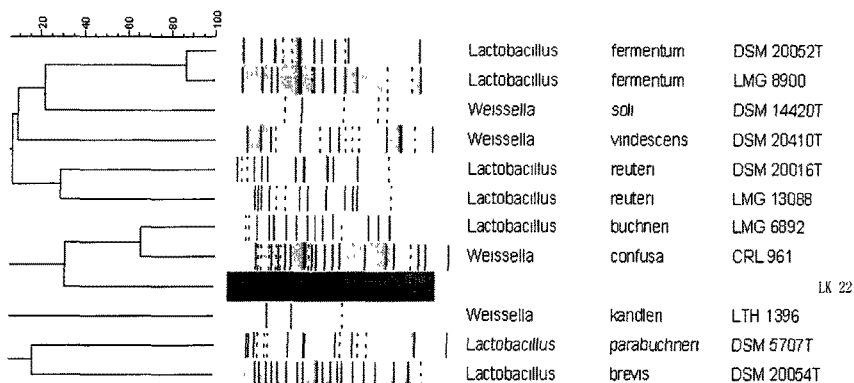


Fig. 3. Rep-PCR fingerprint clustering of obligately heterofermentative rod-shaped *Lactobacilli* reference clusters and Kimchi strains.

Table 2. Antigenotoxic effect of *Leuconostoc* strains

No	Control		Result	DNA Repair (%)	
	Negative	Positive			
LK 1	2	210	125	*	40
LK 2	1	206	155	*	25
LK 3	1	210	136	*	35
LK 4	2	210	130	*	38
LK 5	2	210	121	*	42
LK 6	2	192	158		18
LK 7	2	210	121	**	42
LK 8	2	210	115	*	45
LK 9	1	210	136	*	35
LK 10	1	206	149	*	28
LK 11	4	217	162	**	25
LK 12	4	217	150	***	31
LK 15	1	206	142	*	31
LK 16	1	210	121	*	42
LK 18	1	206	136	*	34
LK 19	1	210	129	*	39
LK 21	2	193	144	*	25
LK 23	2	190	107	*	44
LK 26	3	215	190		12

NC negative control, PC positive control, RE Result

V. References

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