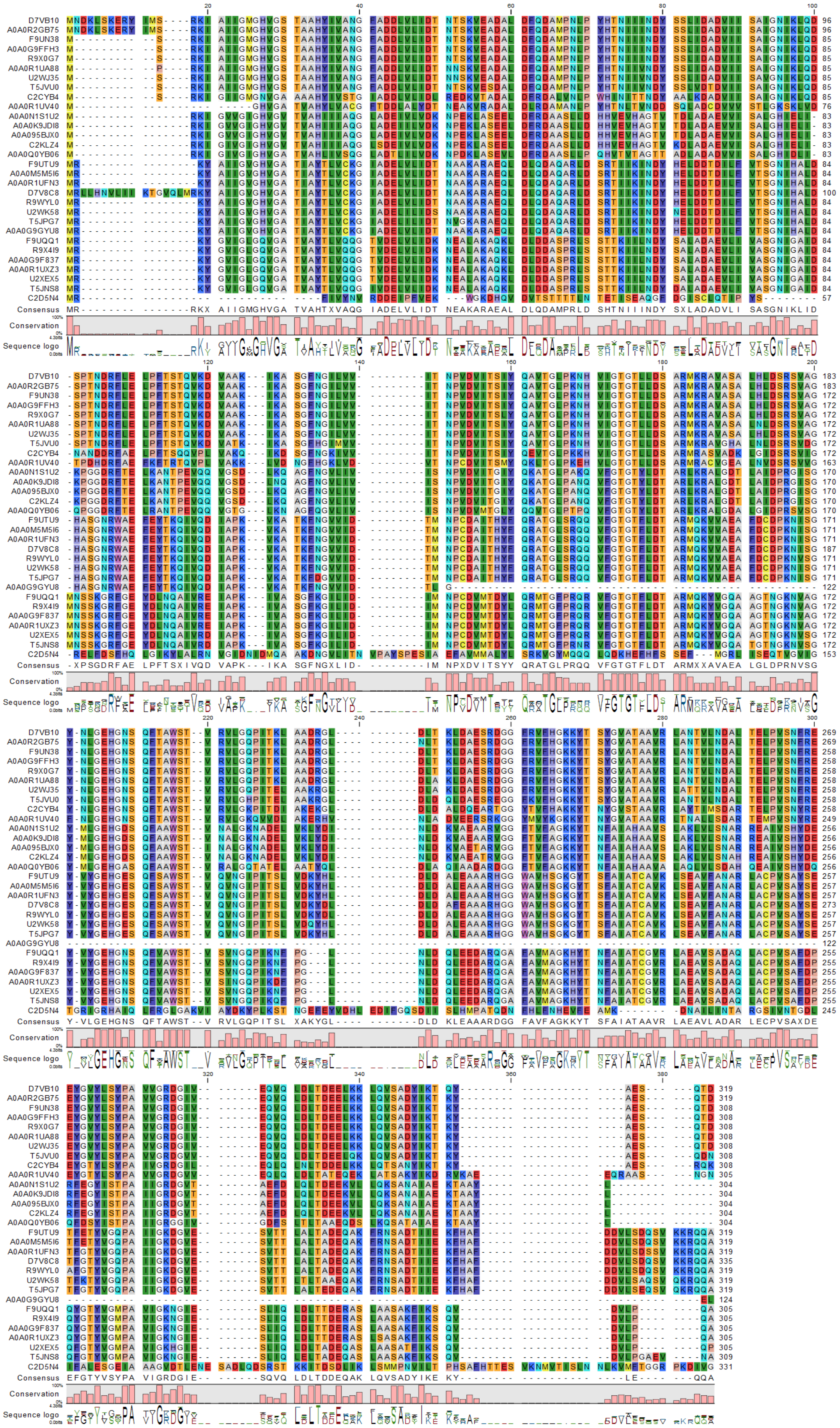
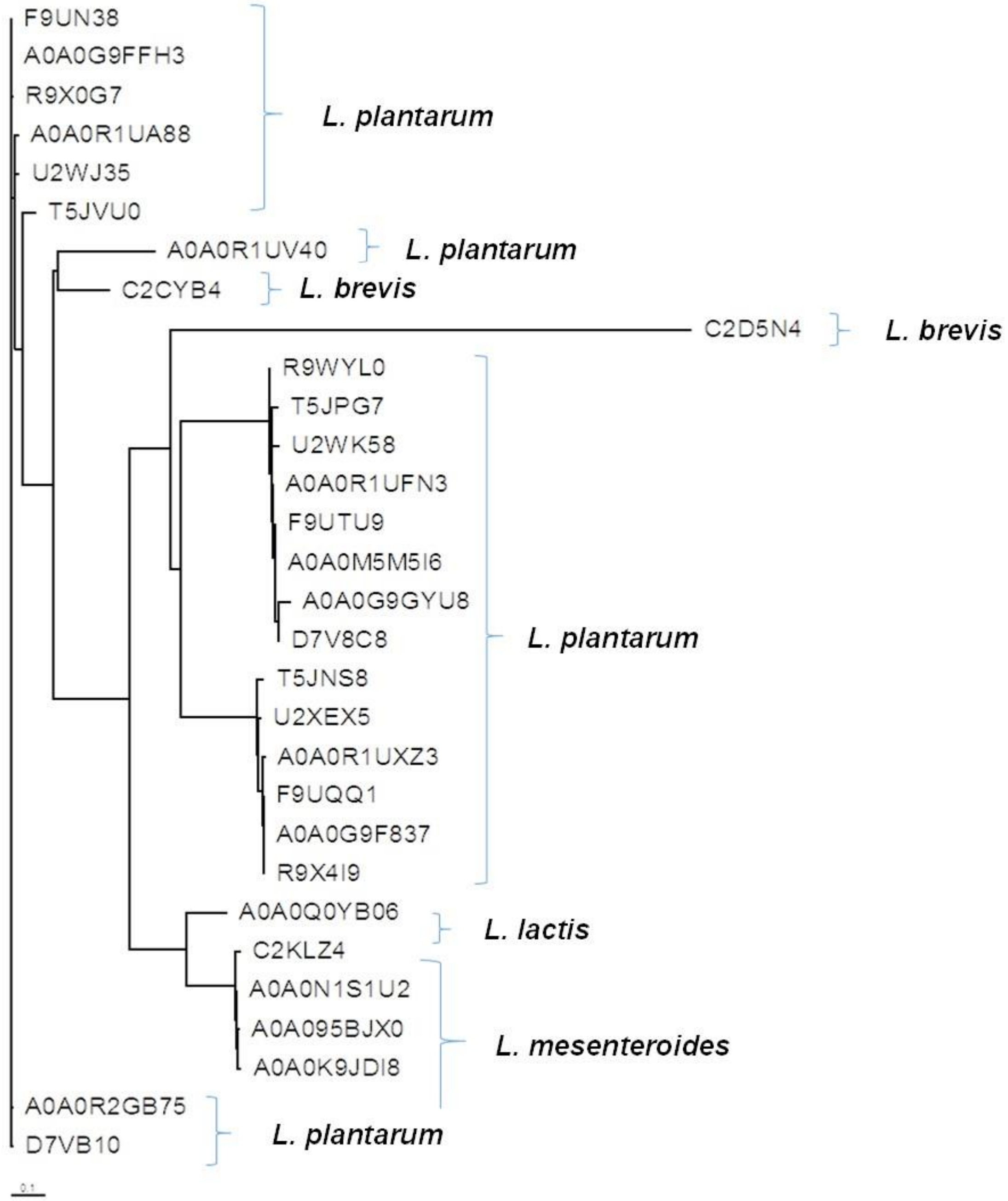
Supplementary information

Identification of 2-hydroxyisocaproic acid production in lactic acid bacteria and evaluation of microbial dynamics during kimchi ripening

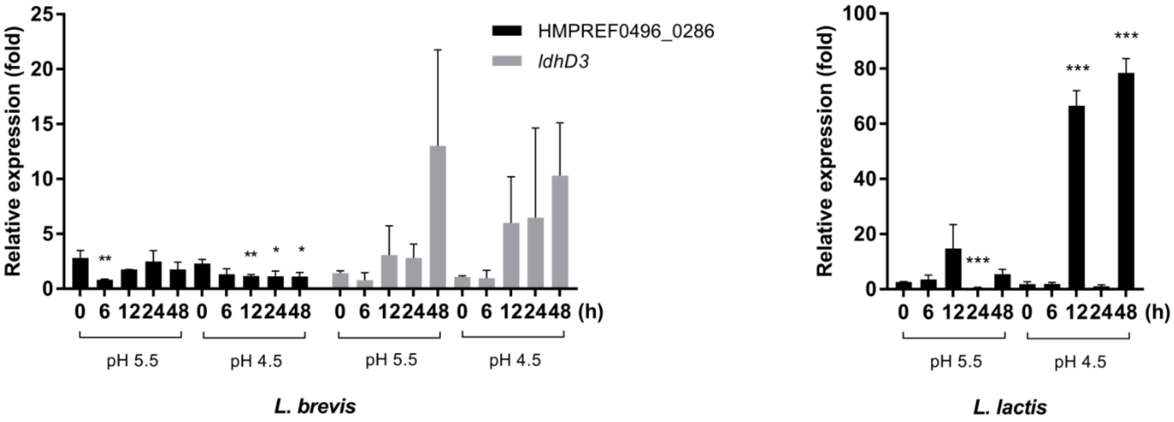
Boyeon Park[†], Hyelyeon Hwang[†], Ji Yoon Chang, Sung Wook Hong, Se Hee Lee, Min Young Jung, Sung-Oh Sohn, Hae Woong Park, and Jong-Hee Lee*



Supplementary figure S1. The multiple sequence alignment of 2-hydroxyisocaproate dehydrogenase of lactic acid bacteria using the ClustalW multi-alignment program



Supplementary figure S2. The phylogenetic trees constructed from 2-hydroxyisocaproate dehydrogenase amino acid sequence obtained Uniprot (www.uniprot.org/). The bar indicates 10% sequence divergence.



Supplementary figure S3. The transcription levels of the 2-hydroxyisocaproate dehydrogenase genes in *L. brevis* and *L. lactis* in MRS of pH 5.5 and 4.5 condition were determined via qRT-PCR. The mRNA expression values were normalized by the transcription levels by the bacteria cultivated in MRS (pH 6.2). Asterisks indicate significant differences (**P* < 0.001; ***P* < 0.01; **P* < 0.05).**

Supplementary table S1. Conserved domain structure of HicD identified in NCBI search

Amino acid sequence of HicD of *Leuconostoc lactis* was compared BLASTP database

Name	Accession	Description	Interval	E-value
HicDH_like	cd05291	L-2-hydroxyisocapronate dehydrogenases and some bacterial L-lactate dehydrogenases	3-304	6.39 E-149
Ldh_1_N	pfam00056	lactate/malate dehydrogenase, NAD binding domain	4-145	4.04 E-30
L-LDH-NAD	TIGR01771	L-lactate dehydrogenase	7-299	5.50 E-96
Mdh	COG0039	Malate/lactate dehydrogenase	3-299	1.32 E-85
ldh	PRK00066	L-lactate dehydrogenase	1-300	3.45 E-73

Supplementary Table S2. The primers for the real-time RT-PCR

Species	Primers	Sequence	Reference
Lactobacillus plantarum	LPWCFS_hicD1	5’-ATTCACGCGTTAGACCATGC-3’ 5’-CTGTTGGCGTGATAATCCCG-3’	In this study
	LPWCFS_hicD2	5’-GCGACGTCTTTGACTTGTGT-3’ 5’-CTTTGCCGATGACCTTGTCC-3’	
	LPWCFS_hicD3	5’-ATCGGACACCACAAGTAGCA-3’ 5’-GTATGTCGGACAAGCAGCTG-3’	
Lactobacillus brevis	HMPREF0496_0286	5’-GATTGGACTTGGATGCGCTT-3’ 5’-TGCTCTAAAATGCCGTCACG-3’	In this study
	Brevis ldhD3	5’-TTGGCGTTGAGAAATGTCGG-3’ 5’-TTCACTGATTAGACGCCCCA-3’	
Leuconostoc lactis	Lactis_AN225_00170	5’-AAGACACCTTGAAAACCCGC-3’ 5’-TTGATTTTCGTGATGCCGCT-3’	In this study
Leuconostoc mesenteroides	LM _LH61_00190	5’-GCGTGTACTTCGACATGATG-3’ 5’-TTGTTGGCATTGGACATGTTG-3’	In this study
Lactobacillus plantarum *Lactobacillus brevis* *Leuconostoc lactis*	16S rRNA	5’-AGCAGTAGGGAATCTTCCA-3’ 5’-CAC CGC TAC ACA TGG AG-3’	Walter *et al.* (2001) Heilig *et al.* (2002)
Leuconostoc mesenteroides	16S rRNA	5’-AGC GTT ATC CGG ATT TAT TG-3’ 5’-CTA CGC ATT CCA CCG CTA CA-3’	Minghui Yan *et al.* (2016)