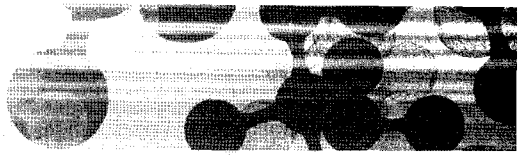

S7-4

Genome analysis of Leuconostoc *kimchii* IMSNU11154

Jongsik Chun, Hyun-Myung Oh, Yong-Joon Cho, Gajin Jeong, Sa-Ouk Kang, Jung-Hye Roe

School of Biological Sciences, Seoul National University, Seoul, Korea

Leuconostoc *kimchii* IMSNU11154 was isolated from kimchi and had been previously identified by polyphasic taxonomy including 16S rDNA sequencing and DNA-DNA hybridization. Kimchi, a true traditional representative of fermented vegetable foods in Korea, has become a rich source of microbial flora for lactic acid bacteria (LAB). With the aid of high throughput sequencing facilities for whole genome shot-gun sequencing procedure and consequent bioinformatics resources were useful in putting the raw genome sequences together into contigs, of which the ends were polished and made seamless by combinatorial PCR and fosmid primer walking. Five plasmids and a single circular chromosomal DNA sequences were assembled for *L. kimchii* IMSNU11154. The circular 2,101,787 bp chromosome and accompanying extrachromosomal plasmids had 37.91% G+C and it contained 2,205 Glimmer-predicted ORFs which covered 89.5 % (1,880,952bp) all over the genomic sequence. Each open reading frame had a mean length of 853 bp. Origin of replication was identified by GC-Skew analysis while 4 copies of rRNA and 68 tRNA genes were analyzed by BLAST and tRNAScanSE.

IMSNU11154 had all the enzymes that take part in Embden-Meyerhof-Parnas Pathway except for fructose 1,6-bisphosphatase [EC: 3.1.3.11] that may be substituted by 1-phosphofructokinase [EC:2.7.1.56]. L-Lactae dehydrogenase [EC:1.1.2.3] and D-lactate dehydrogenase[EC:1.1.1.28] were present as well as malate/lactate dehydrogenase[EC:1.1.1.27] which can also form L-lactate from pyruvate. Transaldolase was missing in Pentose phosphate pathway, the lack of transaldolase can be compensated by phosphoketolase [EC: 4.1.2.9] and D-erythrose-4-phosphate could be utilized for aromatic amino acid and Vitamin B6 metabolisms. Enzymes in Entner-Doudroff pathway (ED pathway) were missing from the genome of this kimchi-microbe but phosphoketolase pathway existed.

Leuconostoc kimchii IMSNU11154, an aerotolerant fermentative anaerobe, had many genes that may be missing in other LAB, and it may require limiting factors or nutrients for its optimal growth requirement. Completion of its genome and subsequent comparative studies with other finished other LAB genomes will reveal its ecological niche or its pivotal roles to play in fermentation processes of kimchi and/or dairy products. And it will help develop a useful probiotic and antibacterial agents of important value in public health.

We have constructed DNA-chips based on the genome sequence obtained in this study. The microarray contained the PCR-amplified DNA fragments which covered 1,868 ORFs and a number of control sequences. The expression profiles under various stress conditions such as heat, osmotic shock, pH change and oxidative stress were examined. The numbers of gene expressed more than three-fold were 75, 35, 24, and 57 under heat, osmotic, acid and oxidative stress conditions, respectively. The expression levels of the majority of genes at osmotic and acid stress conditions were similar. The genes in responsive to oxidative stress contained several thioredoxins and thiol-oxidoreductases. These include two protein disulfide isomerases and a thioredoxin reductase. There are some genes highly expressed under all stress conditions, suggesting a general regulator to control the responses against various stresses. *L. kimchii*, an aerotolerant fermentative anaerobe showed various transcriptional profiles against several environmental stresses. These include some genes related well-known regulatory mechanisms to each condition, but the function of other induced genes remains to be elucidated. Commonly induced by these stress conditions, an unknown putative transcriptional regulator may play an important role in general stress response.