J. Dairy Sci. 97:7363–7372
http://dx.doi.org/10.3168/jds.2014-8299

Transcriptomic analysis of *Lactococcus chungangensis* sp. nov. and its potential in cheese making

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ABSTRACT

Lactococcus lactis has a played a prominent role in food industry from traditional milk fermentations to industrial scale processes. Extensive studies on the biochemical, physiological, and genetic aspects of *L. lactis* are evident from published literature. Recently, another novel species, *Lactococcus chungangensis*, was isolated from activated sludge as the sixth member of the genus to be discovered. To date, no study has been conducted to explore the functional aspects of *L. chungangensis* to identify features similar to those in *L. lactis* that are relevant to the dairy industry. Hence, the present study was undertaken to identify functional genes relevant to dairy application through comparative transcriptomic analysis of *L. chungangensis* with *L. lactis*. In expression microarray data, 415 genes were upregulated and 1,500 were downregulated of the total 1,915 probes analyzed. Interesting findings from this study were the identification of functional genes such as aminohydrolase and S-adenosylmethionine in *L. chungangensis*, which are useful in flavor production in cheese making. Probing these genes by PCR and analyzing the sequence confirmed the presence of these genes. Phenotypic analysis of these genes was also investigated by growing the strain in different concentrations of skim milk media to confirm the ability of *L. chungangensis* to degrade casein in milk, which is the major precursor for flavor enhancing compounds. Other adaptive and stress-response genes such as cold shock and heat shock proteins were also revealed. All experimental investigations at the functional level suggest that *L. chungangensis* possesses some interesting genes which are of commercial significance, especially in cheese production.

Key word: *Lactococcus chungangensis*, transcriptomics, MetC gene, CysK gene, cheese

INTRODUCTION

Dairy lactic acid bacteria (**LAB**) play an important role in dairy industry and are known for their generally recognized as safe status in producing healthy and flavorsome foods. Hence, LAB have been studied extensively to date to enhance both fundamental and application-based knowledge. Members of the *Lactococcus* genus are noted for their ability to produce the L-lactic acid enantiomer from glucose (Schleifer et al., 1985). The genus *Lactococcu*s belongs to the family *Streptococcaceae*, and consists of 9 validly described *Lactococcus* species (http://www.bacterio.net/lactococcus.html). *Lactococcus lactis* is the type species of the genus, which has been routinely used in dairy industry in the production of cheese, yogurt, sour cream, and fermented milk (Cretenet et al., 2011). In cheese making, *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* are used as starter cultures in several types of cheese such as cream cheese, Cheddar cheese, and Swiss cheese (Leroy and De Vuyst, 2004). Other species in the genus such as *L. raffinolactis* also play an important role in making Moroccan soft white cheese (Ouadghiri et al., 2005) and Egyptian Domiati cheese (El-Baradei et al., 2007).

Lactic acid bacteria degrade proteins and use several metabolic routes in producing flavor compounds while they thrive in milk. Thus, their metabolic end products and enzymes have significant influence in determining the texture and flavor of the final product (Samaržija, 2011; Kim, 2014). One of the main precursors for flavor compounds in milk is casein, a milk protein that is also derived from fatty acids and sugars (Liu et al., 2008). The proteolytic system of LAB degrades casein into different amino acid constituents such as methionine and cysteine, which are sulfur-containing amino acids. The volatile sulfur compounds such as methanethiol and hydrogen sulfide, the odor compounds in several types of cheese are the end products of metabolism of methionine and cysteine in dairy bacteria (Bogicevic et al., 2012). S-Adenosylmethionine synthase is one such enzyme which is involved in methionine catabo-

Received April 28, 2014.
Accepted September 4, 2014.
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lism, forming a precursor to methanethiol, one of the primary flavor compounds found in Cheddar cheese (Weimer et al., 1999) besides playing a role in other metabolic processes and general maintenance of cells. S-Adenosylmethionine is derived from the pathways of methionine and cysteine biosynthesis pathways in lactic acid bacteria (Irmler et al., 2009).

Lactococcus chungangensis CAU 28^T was isolated from activated sludge and characterized against 5 recognized species by comparing phenotypic properties and cellular fatty acid composition, and by 16S rRNA gene sequencing (Cho et al., 2008). Phenotypic characterization alone was not enough to delineate *L. chungangensis* CAU 28^T from other *Lactococcus* species. The major fatty acid components were saturated fatty acids C16:0 and C14:0 and unsaturated fatty acid C18:1v7c; however, the fatty acid profile of this strain was similar to those of other reference *Lactococcus* type strains except *Lactococcus piscium* DSM 6634^T. Genomic studies are the way forward to delineate this species and also to understand its unique characteristics. Until now, this strain has not been subject to genomic characterization, which has the potential to identify genes that are specific to this strain and also of commercial interest, especially in cheese making.

Whole-genome sequencing to elucidate the genetic content of a microorganism is considered to be an expensive and time-consuming procedure. Approaches that alleviate the necessity to sequence a large number of genes and that identify unique and common genes without sequencing are a trend in the present era. High-throughput microarrays are one such technology by which it is easier to identify both favorable and unfavorable genes. So far, gene expression microarrays are commonly used to determine the genomic content of bacteria, including inter-species comparison (Hakenbeck et al., 2001; Fukiya et al., 2004; van Hijum et al., 2008; Park et al., 2012). Gene expression microarray analysis is also widely used to answer important biological questions at a molecular level that are not easily answered using other detection techniques (dos Reis et al., 2003; Joo et al., 2011).

In the dairy industry, genome-wide comparisons of both starter and nonstarter LAB have been done, but no studies at the transcriptome level have been reported. A strategy was adopted in the current study to identify the functional genes of commercial significance without the need to sequence the whole genome. Gene expression analysis was done on *L. chungangensis* by in vitro transcriptome microarray experiments using an open reading frame microarray platform based on the *L. lactis* ssp. *lactis* Il1403 platform.

MATERIALS AND METHODS

Cultivation of Bacteria, RNA Extraction, and cDNA Synthesis and Labeling

Lactococcus chungangensis CAU 28^T was grown along with the reference strain *L. lactis* ssp. *lactis* KCTC 3769^T on tryptic soy agar (**TSA**; Becton Dickinson, Sparks, MD) at 30°C for 18 h. RNeasy Mini kit (Qiagen, Valencia, CA) was used to extract total RNA. The concentration of RNA was checked by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and standardized. cDNA was synthesized from RNA by reverse transcription with the SuperScript double-stranded cDNA synthesis kit using an oligo dT primer from cDNA synthesis kit (Invitrogen Life Technologies, Carlsbad, CA). Briefly, 10 μg of total RNA was reverse-transcribed to cDNA using an oligo dT primer, then second-strand cDNA synthesis was performed. After purification, the cDNA was quantified using the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). The cDNA was labeled using the One-Color Labeling Kit (Nimblegen, Madison, WI) according to the Nimblegen expression protocol. One microgram of each cDNA sample was labeled with Cy3 using Cy3-random nonamer. After purification, the labeled cDNA was quantified using the NanoDrop ND-1000 spectrophotometer.

Microarrays, Data Acquisition, and Statistical Analysis

The custom microarray *L. lactis* pangenome 385K short oligo array (Nimblegen), with 2,321 genes: 735,308 probes, and 5 replicates, was mapped onto the whole-genome data from *L. lactis* ssp. *lactis* Il1403 (GenBank accession number NC_002662.1) and whole-genome scaffolds for *L. chungangensis* (unpublished data). The free energy of hybridization, Go, of probes to cDNA was calculated using the HyTherTM (SantaLucia, 1998; SantaLucia and Hicks, 2004) web service (http://ozone3.chem.wayne.edu/cgi-bin/login/login/loginVerify.cgi) in 1 × SSC at 42°C with the default concentrations, and the correction for microchips was applied (Fotin et al., 1998). Probes with calculated Go < -45 kcal/mol were treated as giving a full hybridization signal, whereas probes of the draft genome of *L. chungangensis* with Go > -25 kcal/mol were treated as failing to hybridize. Signals from probes with a free energy of hybridization between -25 and -45 kcal/mol were corrected by 15% per kcal/mol. NimbleScan (version 2.5, Gene Expression RMA algotithm) was used to extract raw data and logarithmic transformation (base2) of the

signals was performed at 532 nm with a resolution of 2 μm. The signals on the *L. chungangensis* CAU 28^T were normalized to the *L. lactis*, based upon the signal levels of a set of structural ribosomal proteins, by 0.2 × the corrected signal levels. An increase in normalized signal level of at least 2-fold was used to determine upregulated gene expression. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity, as shown in Park et al. (2012). The analyzed data has been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and is accessible through GEO series accession number GSE58002 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58002).

PCR Amplification of MetC and CysK Genes

Genomic DNA from *L. chungangensis* CAU 28^T and *L. lactis* ssp. *lactis* KCTC 3769^T were extracted by i-genomic BYF DNA Extraction mini kit (iNtRON Biotechnology, Seoul, Korea). Primer sets were designed targeting methionine and cysteine biosynthesis including the S-adenosylmethionine synthase pathway involved in sulfur metabolism (Fernandez et al., 2002). Primer sets were designed for cystathionine β-lyase (*MetC*) gene (5′-CTGGAATTCATGCGGTTCTT-3′ and 5′-ACGCCACCTAGTGATTCAGC-3′) and O-acetylserine sulfhydrylase (*CysK*) gene (5′-ACAGAAGTGCATACAATTCA-3′ and 5′-GATTAAGAAGATTAAAGGAGG-3′) using PRIMER3 software (Rozen and Skaletsky, 1999) based on the gene sequence of *L. lactis* ssp. *cremoris* MG1363. A PCR reaction mixture (20 μL) consisting of 5 U of Taq DNA polymerase (BEAMS Biotechnology, Seongnam, Korea), 2.0 μL of 10× Taq buffer, 1 μL of dNTP mixture, 1 μL at 10 m*M* of each primer, and 3 μL of genomic DNA template was used for amplification of target genes. Amplifications were performed in a TProfessional Thermocycler (Biometra GmbH, Göttingen, Germany) with an initial denaturation step at 95°C for 5 min, 30 repeated cycles of 95°C for 1 min, annealing 55°C for 1 min and extension at 72°C for 1 min, and a final cycle of extension at 72°C for 10 min. Three microliters of amplified PCR products were electrophoresed on 1.2% Seakem LE agarose gel (FMC Bioproducts, Rockland, ME) and the gel was visualized with Gel DOC XR$^+$ Imaging system (Bio-Rad Laboratories, Hercules, CA). The PCR products were sequenced by using a BigDye Terminator Cycle Sequencing kit and an automatic DNA sequencer (model 3730; Applied Biosystems, Foster City, CA). Sequences were aligned using the CLUSTAL_X 1.81 program (Thompson et al., 1997) with parameters set against corresponding sequences from the NCBI GenBank database. The nucleotide sequences obtained in this study were deposited in GenBank under the accession numbers KM073976 (methionine) and KM073977 (cysteine), respectively.

Growth Conditions in Different Concentrations of Skim Milk Media

The coagulation and proteolysis of casein in milk was tested by adding skim milk powder (Becton Dickinson) to TSA (Becton Dickinson). To compare the proteolytic ability of *L. chungangensis* CAU 28^T, the reference strain *L. lactis* ssp. *lactis* KCTC 3769^T was grown in the same media and incubated at 30°C for 48 h. The growth pattern of *L. chungangensis* CAU 28^T was observed in 5% and 20% liquid skim milk media cultivated aerobically at 30°C in triplicates with a control strain. Cell densities were measured using Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland) spectrophotometer at a wavelength of 600 nm.

RESULTS

Microarray Statistical Analysis

The comparison of the expression profiles by hybridization to the immobilized probes on the microarray of *L. lactis* ssp. *lactis* Il1403 (GenBank accession number NC002662) with the total RNA of *L. chungangensis* CAU 28^T, showed reproducibility with interarray correlation values (range: $-1 \leq r \leq 1$) and pairwise scatter plots of gene expression values ($\log_2$) as shown in Figure 1. A correlation value (r) of 0.29 was found between reference strain *L. lactis* and the tester strain *L. chungangensis*, suggesting a positive correlation between the two. Of the 1,915 genes analyzed, the expression level was upregulated for 415 genes and downregulated for 1,500 genes in *L. chungangensis*. The Euclidean hierarchical clustering method with normalized signal shows upregulated genes in red and downregulated genes in green (Figure 2).

Identification of Upregulated and Downregulated Genes in L. chungangensis

Genes that were upregulated and downregulated were identified, especially those involved in flavor formation in cheese making using a cluster of orthologous groups (COG) approach (ftp://ftp.ncbi.nih.gov/pub/wolf/COGs/COG0303/fun.txt). The majority of up- and downregulated genes belonged to translation and transcription (J and K), DNA replication (L), cellular division and cell envelope (D and M), posttranslational modification (O), energy production (C), carbohydrate transport and metabolism (G), amino acid transport

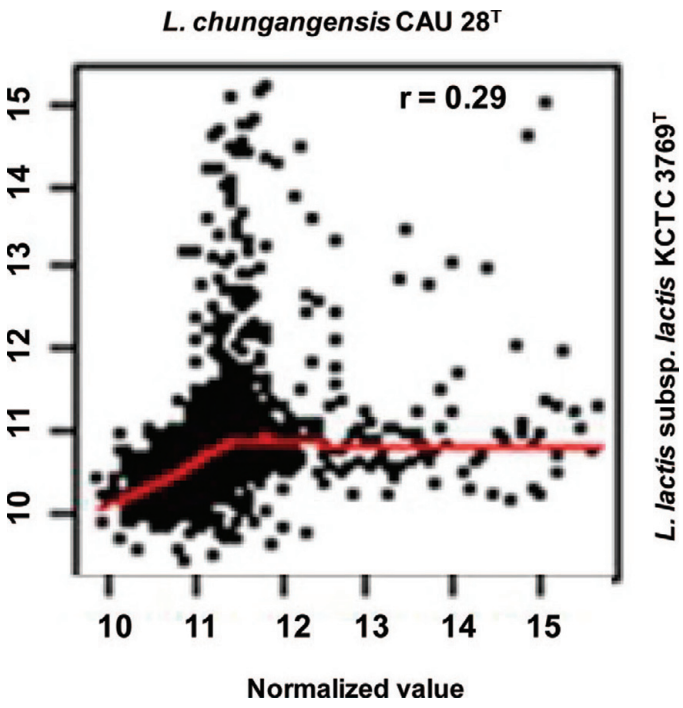

Figure 1. Reproducibility and dynamic range with pairwise scatter plot. Gene expression values are shown in terms of $\log_2$. Color version available in the online PDF.

and metabolism (E), inorganic ion transport (P), and function unknown (S) categories (Table 1). The 39 examples out of 415 upregulated genes that were classified as related to cheese flavor production included amino acid amidohydrolase, S-adenosylmethionine synthase, alanine racemase, and glutamate decarboxylase ,which were classified in amino acid transport and metabolism (E), and glyceraldehyde-3-phosphate dehydrogenase, L-lactate dehydrogenase, and mannitol-specific PTS system IIA component, which were classified in carbohydrate transport and metabolism (G). Interestingly, stress-response genes which were classified in transcription (A) and posttranslational modification, protein turnover, chaperones (O), such as cold shock protein E, a 2-component system regulator, and molecular chaperone DnaK, were also upregulated.

Notable findings from this study were the identification of functional genes such as aminohydrolase and S-adenosylmethionine that were upregulated in *L. chungangensis* which play a role in flavor production, especially S-adenosylmethionine, related to flavor production in cheese through the methionine and cysteine pathway.

The findings also identified 1,500 downregulated genes in *L. chungangensis* CAU 28^T. Thirty-nine examples of downregulated genes were also classified using the cluster of orthologous groups approach (Table 1). Genes were classified in amino acid transport and metabolism (E), such as peptide binding protein and intercellular adhesion protein. Ferrous ion transport protein A, multidrug efflux transporters, and cationic amino acid transport were classified in inorganic ion transport and metabolism (P). Genes were also classified in carbohydrate transport and metabolism (E), such as β-glucosidase and phospho-β-glucosidase. Stress-response genes such as NADH oxidases were classified in energy production and conversion (C). The 39 examples of up- and downregulated genes shown in Table 1 were related with hierarchical mapping (Figure 2) showing the relative level of expression; red represents high expression.

Sequencing Results of Cheese-Making Flavor Genes

The cystathionine β-lyase (*MetC*) and O-acetylserine sulfhydrylase (*CysK*) genes are important factors in determining the flavor-enhancing aspects in *L. chungangensis*. Sequencing the *MetC* and *CysK* genes of the *L. chungangensis* CAU 28^T strain shows that *MetC* is 684 bp and *CysK* is 927 bp in length. The sequence identities of the *MetC* and *CysK* genes of the *L. chungangensis* CAU 28^T strain were 63.7 to 66.6% for *L. lactis* ssp. *lactis* and 69.3 to 66.8% for *L. lactis* ssp. *cremoris*.

Phenotypic Analysis of Functional Genes in L. chungangensis and Their Application in Cheese Making

Phenotypic analysis of the coagulation and proteolysis of casein in *L. chungangensis* CAU 28^T was investigated. The strain can grow on skim milk media up to 20% as indicated by the clear zone on the TSA-skim milk agar plates (Figure 3). Proteolytic bacteria can hydrolyze casein to form soluble nitrogenous compounds, as indicated by the clear zone surrounding the colonies. The growth pattern in both 5% and 20% skim milk media showed no exponential growth, although higher cell densities were noted in 20% skim milk media (Figure 4). No significant difference was found in the growth patterns of *L. chungangensis* CAU 28^T and *L. lactis* ssp. *lactis* KCTC 3769^T in skim milk media.

DISCUSSION

Lactic acid bacteria play an essential role in the food industry with their notable metabolism, contributing to the sensory characteristics of the products and inhibiting the growth of undesirable microorganisms, ensuring the quality and safety of the final product. Extensive research has been done on this group of microbes, which

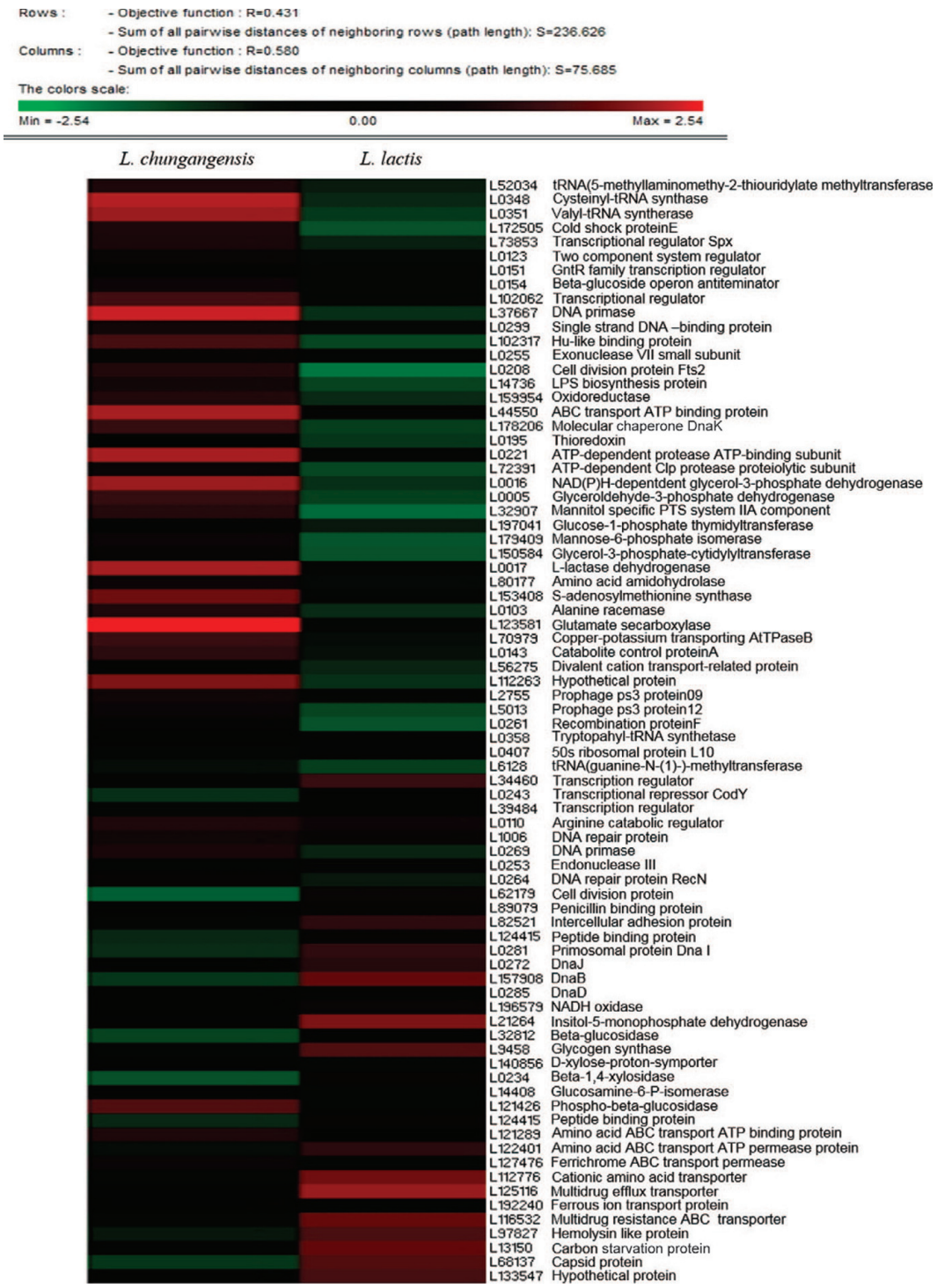

Figure 2. Hierarchical map of differentially expressed genes in cheese-making flavor functions. Black (red) represents high expression; gray (green) represents low expression. Color version available in the online PDF.

includes isolation of novel strains which are adaptable, robust, and have superior qualities to existing strains. The current study is aimed at identifying such novel properties with the potential to enhance the quality of the product in cheese making

Comparative transcriptomics was performed on this strain with the reference strain *L. lactis* ssp. *lactis* KCTC 3769^{T}. Global transcriptomic analysis of both strains shows a low relationship of *L. chungangensis* CAU 28^{T} with *L. lactis* ssp. *lactis* KCTC 3769^{T}. This low

Table 1. List of 39 upregulated and downregulated genes involved in cheese-making flavor in *L. chungangensis* CAU 28^T using a cluster of orthologous group approach

Category	Gene name	Description	Expression
Information storage and processing			
Translation, ribosomal structure, and biogenesis (J)	L52034	tRNA(5-methyllaminomethy-2-thiouridylate methyltransferase	Up
	L0348	Cysteinyl-tRNA synthase	Up
	L0351	Valyl-tRNA synthetase	Up
Trancription (K)	L172505	Cold shock protein E	Up
	L73853	Transcriptional regulator Spx	Up
	L0123	Two-component system regulator	Up
	L0151	GntR family transcription regulator	Up
	L0154	β-Glucoside operon antiteminator	Up
	L102062	Transcriptional regulator	Up
DNA replication, recombination, and repair (L)	L37667	DNA primase	Up
	L0299	Single-strand DNA-binding protein	Up
	L102317	Hu-like DNA-binding protein	Up
	L0255	Exonuclease VII small subunit	Up
Cellular processes			
Cell division and chromosome partitioning (D)	L0208	Cell division protein Fts2	Up
Cell envelope biogenesis, outer membrane (M)	L14736	LPS biosynthesis protein	Up
	L159954	Oxidoreductase	Up
	L44550	ABC transport ATP binding protein	Up
Posttranslational modification, protein turnover, chaperones (O)	L178206	Molecular chaperone DnaK	Up
	L0195	Thioredoxin	Up
	L0221	ATP-dependent protease ATP-binding subunit	Up
	L72391	ATP-dependent Clp protease proteolytic subunit	Up
Metabolism			
Energy production and conversion (C)	L0016	NAD(P)H-dependent glycerol-3-phosphate dehydrogenase	Up
Carbohydrate transport and metabolism (G)	L0005	Glyceraldehyde-3-phosphate dehydrogenase	Up
	L32907	Mannitol-specific PTS system IIA component	Up
	L197041	Glucose-1-phosphate thymidyltransferase	Up
	L179409	Mannose-6-phosphate isomerase	Up
	L150584	Glycerol-3-phosphate-cytidylyltransferase	Up
	L0017	L-Lactate dehydrogenase	Up
Amino acid transport and metabolism (E)	L80177	Amino acid amidohydrolase	Up
	L153408	S-Adenosylmethionine synthase	Up
	L0103	Alanine racemase	Up
	L123581	Glutamate decarboxylase	Up
Inorganic ion transport and metabolism (P)	L70979	Copper-potassium transporting ATPase B	Up
	L0143	Catabolite control protein A	Up
	L56275	Divalent cation transport-related protein	Up
Poorly characterized			
Function unknown (S)	L112263	Hypothetical protein	Up
	L2755	Prophage ps3 protein09	Up
	L5013	Prophage ps3 protein12	Up
	L0261	Recombination protein F	Up
Information storage and processing			
Translation ribosomal structure and biogenesis (J)	L0358	Tryptophanyl-tRNA synthetase	Down
	L0407	50S ribosomal protein L10	Down
	L6128	tRNA(guanine-N-(1)-)-methyltransferase	Down
Transcription(K)	L34460	Transcription regulator	Down
	L0243	Transcriptional repressor CodY	Down
	L39484	Transcription regulator	Down
	L0110	Arginine catabolic regulator	Down
DNA replication, recombination, and repair (L)	L1006	DNA repair protein	Down
	L0269	DNA primase	Down
	L0253	Endonuclease III	Down
	L0264	DNA repair protein RecN	Down
Cellular processes			
Cell division and chromosome partitioning (D)	L62179	Cell division protein	Down
Cell envelope biogenesis, outer membrane (M)	L89079	Penicillin binding protein	Down
	L82521	Intercellular adhesion protein	Down
	L124415	Peptide binding protein	Down
Posttranslational modification, protein turnover, chaperones (O)	L0281	Primosomal protein DnaI	Down
	L0272	DnaJ	Down
	L157908	DnaB	Down

Continued

Table 1 (Continued). List of 39 upregulated and downregulated genes involved in cheese-making flavor in *L. chungangensis* CAU 28^T using a cluster of orthologous group approach

Category	Gene name	Description	Expression
	L0285	DnaD	Down
Metabolism			
Energy production and conversion (C)	L196579	NADH oxidase	Down
	L21264	Inosine-5-monophosphate dehydrogenase	Down
Carbohydrate transport and metabolism (G)	L3281	β-Glucosidase	Down
	L9458	Glycogen synthase	Down
	L140856	D-xylose proton-symporter	Down
	L0234	β-1,4-Xylosidase	Down
	L14408	Glucosamine-6-P-isomerase	Down
	L121426	Phospho-β-glucosidase	Down
Amino acid transport and metabolism (E)	L124415	Peptide binding protein	Down
	L121289	Amino acid ABC transport ATP binding protein	Down
	L122401	Amino acid ABC transport ATP permease protein	Down
	L127476	Ferrichrome ABC transport permease	Down
Inorganic ion transport and metabolism (P)	L11277	Cationic amino acid transporter	Down
	L125116	Multidrug efflux transporter	Down
	L192240	Ferrous ion transport protein A	Down
	L116532	Multidrug resistance ABC transporter	Down
Poorly characterized			
Function unknown (S)	L97827	Hemolysin-like protein	Down
	L13150	Carbon starvation protein	Down
	L68137	Capsid protein	Down
	L133547	Hypothetical protein	Down

correlation was observed at least because of sequence divergence of *L. chungangensis* CAU28^T from *L. lactis* ssp. *lactis* KCTC 3769^T. The multiple probes per gene on the Nimblegen platform mean that probes to more conserved regions in the genes still hybridize successfully. Of the 415 upregulated genes in *L. chungangensis* CAU 28^T, functions relating to protein metabolism, such as amino acid aminohydrolase, alanine racemase, and glutamate decarboxylase, are noteworthy. These genes are a part of the proteolytic system in LAB and

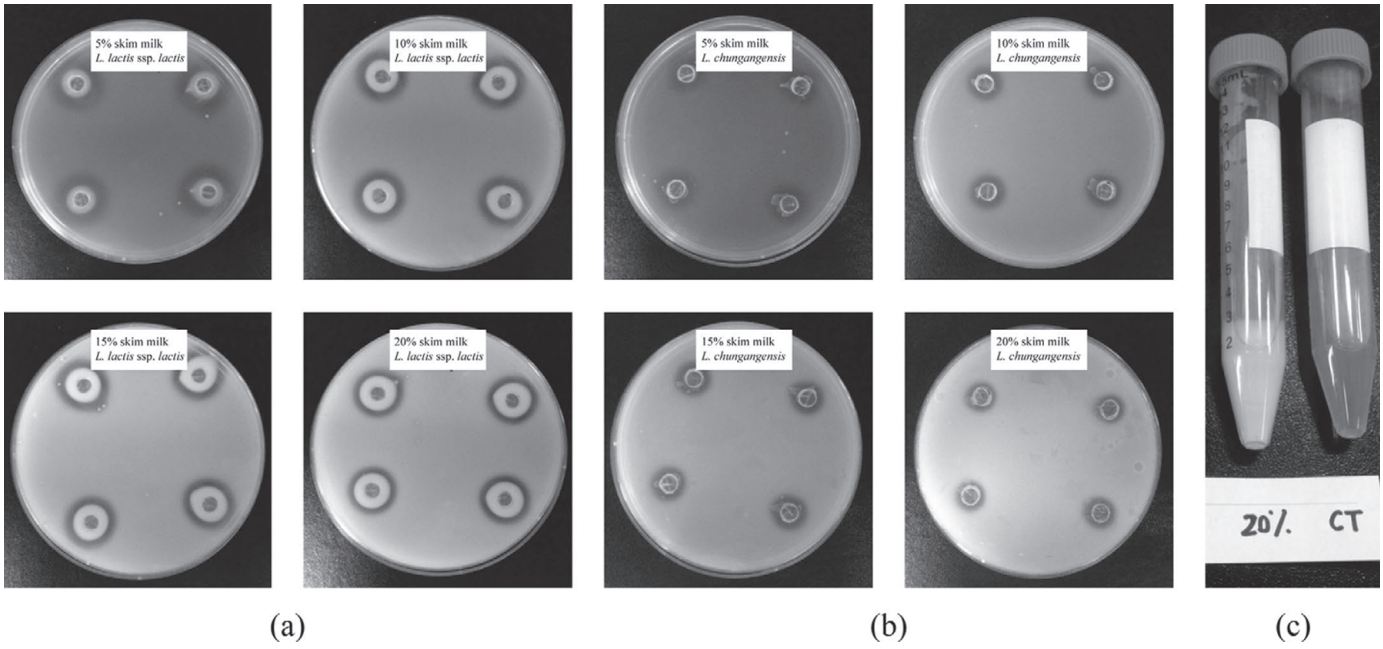

Figure 3. (a) The growth of *Lactococcus lactis* ssp. *lactis* KCTC 3769^T and (b) *Lactococcus chungangensis* CAU 28^T on tryptic soy agar with 5%,10%, 15%, and 20% skim milk agar plates, and (c) coagulation of *L. chungangensis* CAU 28^T in 20% skim milk media in comparison with a noncoagulating control strain (CT).

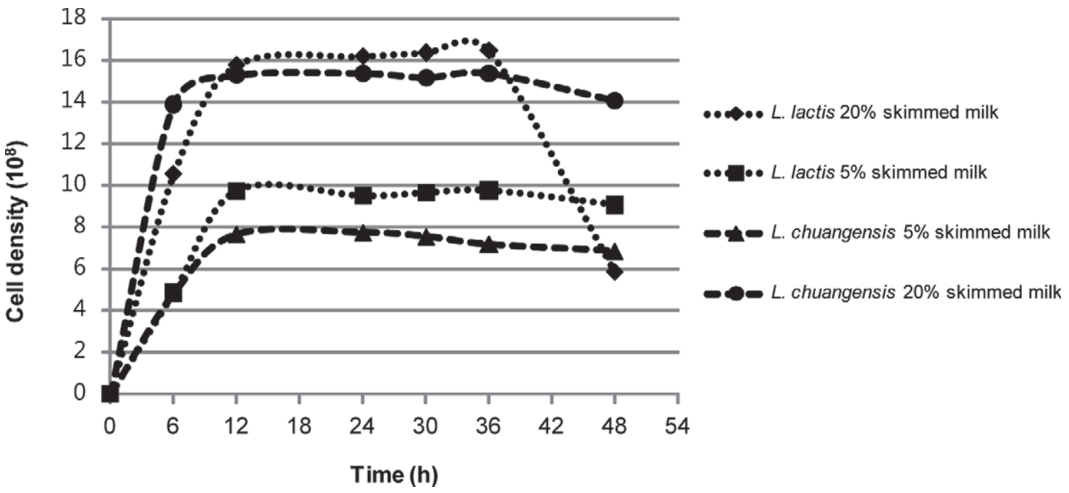

Figure 4. Physiological growth pattern of *Lactococcus chungangensis* CAU 28^T and *Lactococcus lactis* ssp. *lactis* 3769^T in skim milk media.

are involved in biosynthesis and catabolism of proteins into amino acids and peptides, and have proven to be crucial for cheese ripening.

In cheese making, strains that have the capacity to grow up to high cell densities producing lactic acid and to ferment milk carbohydrates are of commercial importance. Milk is a very complex medium that changes continuously during fermentation. It consists of the milk protein casein that is degraded by the bacterial proteases into peptides and amino acids, as well as vitamins (de Jong et al., 2013). At high cell densities, amino acids in milk may not suffice for the entire growing population of cells. Therefore, the alternative is to resort to extracellular hydrolysis of large proteins such as casein into short peptides and amino acids before transport for use as flavor precursors in cheese production (Xie et al., 2004).

Lactococci have such a proteolytic system, which is the key for flavor formation in cheese (Urbach, 1995). The hydrolyzed amino acids also act as precursors for a multitude of volatile compounds. The growth of *L. chungangensis* CAU 28^T in 20% skim milk media demonstrates that this organism can degrade casein in milk and produce acid from fermentable carbohydrates, proving it to be useful in proteolysis of cheese ripening, contributing to the texture and flavor of cheese. Higher cell densities were recorded in 20% compared with 5% skim milk media, supporting the presence of enzymes necessary for hydrolysis of proteins. One of the functional genes expressed in *L. chungangensis* CAU 28^T is S-adenosylmethionine synthase, which is involved in methionine catabolism. It is a precursor to methanethiol, one of the primary flavor compounds found in Cheddar cheese (Weimer et al., 1999) besides playing a role in other metabolic processes and general maintenance of the cell.

Furthermore, identification of *MetC* and *CysK* genes supports the flavor-enhancing capacity of *L. chungangensis* CAU 28^T, as both genes are a part of cysteine and methionine metabolism pathways that lead to the formation of volatile sulfur compounds such as methanethiol, which is the dominant odor-active compound in many varieties of cheese (Bogicevic et al., 2012).

Compared with gene sequences with *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris*, which play important roles in the processing of cheese (Leroy and De Vuyst, 2004), the identities among species were over 60% in both of *MetC* and *CysK* genes. Generally, it is known that it is difficult to find the differences at the interspecies level of microorganisms using cDNA microarray hybridization (Aguado-Urda et al., 2010). However, our finding is interesting in that transcriptomic microarray analysis could successfully identify functional genes without laborious whole-genome sequencing analysis for industrial purposes.

In the present study, upregulated genes relating to carbohydrate and sugar metabolism, such as multiple sugar ABC transporters are common to most bacteria, not only LAB. These transporters might have specificity to oligosaccharides such as raffinose and are known to transport multiple substrates.(Ajdic and Pham, 2007).

As revealed by transcriptomic analysis, *L. chungangensis* also harbors stress-response genes such as cold shock protein E, superoxide dismutase, and 2-component system regulator. Such stress-response genes are useful in cheese making, as strains are subjected to acid, cold, and heat stress (Xie et al., 2004). For example, cold shock protein E prevents protein misfolding during cold stress, whereas superoxide dismutase activity helps combat acid stress, as revealed by previous studies in acid stress-induced protein expression in *L. lactis* (Sanders et al., 1995). Similarly, heat shock

proteins are involved in the maturation of newly synthesized proteins, proper refolding, or degradation of denatured protein (Georgopoulos and Welch, 1993; Hartl et al., 1994). Most organisms respond to heat shock by synthesizing a conserved set of proteins, such as the DnaK-DnaJ-GrpE and GroEL-GroES chaperone complex (Yura et al., 1993). In contrast, the NADH oxidase common oxidative stress resistance mechanism found in *L. lactis* (Miyoshi et al., 2003) was absent in *L. chungangensis*.

In comparison with *L. lactis*, some undesirable genes were downregulated in *L. chungangensis* CAU 28^{T}, such as multidrug efflux transporter and multidrug resistance ABC transporter, which usually play a major role in intrinsic resistance in gram-negative bacteria (Levy, 1992). The latter are considered to form pores in the cytoplasmic membrane of erythrocytes, leukocytes, and other cells, leading to modification of cellular functions, lysis of host cells, or both (Sakiyama et al., 2006). The absence of such genes in *L. chungangensis* makes it more desirable for food fermentation.

Functional genes involved in degradation of casein, tolerance to stress conditions, and ability to use a variety of substrates were revealed by transcriptomic analysis of *L. chungangensis* CAU 28^{T}, proving it to be useful as a starter or nonstarter strain in cheese manufacturing. Further investigation on the ability of the strain to inhibit the growth of other food spoilage organisms should make it economically more viable. This pilot study should lead to adaptation of the strain, probing the physiology further for fermentation at laboratory and industrial scales.

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