Indian J Microbiol (July–Sept 2014) 54(3):293–301
DOI 10.1007/s12088-014-0456-x

ORIGINAL ARTICLE

Comparative Genomic Analysis of Two-Component Signal Transduction Systems in Probiotic *Lactobacillus casei*

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Received: 15 October 2013 / Accepted: 5 February 2014 / Published online: 14 February 2014

Abstract *Lactobacillus casei* has traditionally been recognized as a probiotic, thus needing to survive the industrial production processes and transit through the gastrointestinal tract before providing benefit to human health. The two-component signal transduction system (TCS) plays important roles in sensing and reacting to environmental changes, which consists of a histidine kinase (HK) and a response regulator (RR). In this study we identified HKs and RRs of six sequenced *L. casei* strains. Ortholog analysis revealed 15 TCS clusters (HK–RR pairs), one orphan HKs and three orphan RRs, of which 12 TCS clusters were common to all six strains, three were absent in one strain. Further classification of the predicted HKs and RRs revealed interesting aspects of their putative functions. Some TCS clusters are involved with the response under the stress of the bile salts, acid, or oxidative, which contribute to survive the difficult journey through the human gastrointestinal tract. Computational predictions of 15 TCSs were verified by PCR experiments. This genomic level study of TCSs should provide valuable insights into the conservation and divergence of TCS proteins in the *L. casei* strains.

Keywords Comparative genomics · *Lactobacillus casei* · Probiotic · Two-component signal transduction system

Introduction

Lactobacillus spp. have achieved popularity in the manufacturing of probiotic products because of their convincing beneficial effects on human health. However, before providing benefit to human health, probiotic bacteria must survive the industrial production processes and transit through the gastrointestinal tract [1, 2]. Bacterial two-component signal transduction systems (TCSs) play important roles for many bacteria by enabling them to detect and respond to diverse changes/stresses in the environment [3]. TCS genes are typically located within the same operon encoding two signalling proteins: a transmembrane sensor histidine kinase (HK) and a cytoplasmic response regulator (RR), which may sometimes be carried by a single polypeptide to form the hybrid HKs [3].

Individual HKs contain a conserved kinase core and respond to environmental signals by autophosphorylation of a histidine residue, creating a high-energy phosphoryl group, which is then transferred to an aspartate residue in the RRs. The RRs, which are usually transcriptional regulators, contain a conserved regulatory domain. Phosphorylated RRs then activate downstream specific responses [3]. In most HKs, the transmitter domain shows high sequence conservation, especially within a set of six recognizable motifs or boxes designated H, N, F, G1, G2, and G3. In particular, the H box contains an invariant H residue that is autophosphorylated in an ATP-dependent manner [4]. RRs generally contain at least two functional domains: a conserved N-terminal receiver domain (REC domain) that is phosphorylated by the HK at a strictly

Electronic supplementary material The online version of this article (doi:10.1007/s12088-014-0456-x) contains supplementary material, which is available to authorized users.

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Table 1 The information of six sequenced *L. casei* genomes

Organism	Assembly	Chrs	Plasmids	Size (Mb)	GC, %	Gene	Protein	Origin
L. casei ATCC 334	ASM1452v1	1	1	2.92	46.6	2,922	2,764	Emmental cheese
L. casei LC2W	ASM19478v1	1	1	3.08	46.4	3,264	3,164	Dairy products
L. casei BD-II	ASM19476v1	1	1	3.13	46.3	3,300	3,199	Homemade koumiss
L. casei BL23	ASM2648v1	1	–	3.08	46.3	3,072	2,997	Human Feces
L. casei str. Zhang	ASM1924v1	1	1	2.90	46.4	2,949	2,847	Homemade koumiss
L. casei W56	ASM31803v1	1	1	3.13	46.3	3,234	3,126	Dairy products

conserved D residue, and one or more variable C-terminal output domains [5]. Modulation of the phosphorylated state of the RR controls either expression of the target genes or cellular behaviour.

Lactobacillus casei is a facultative heterofermentative lactic acid bacterium. It has traditionally been recognized as a probiotic and used in commercial products for its health-promoting and nutritional properties [6–8]. *L. casei* requires a complex array of TCS proteins to cope with diverse human hosts, host responses, and environmental conditions. The TCS MaeKR belonging to the citrate family is essential for the expression of malic enzyme of *L. casei* strains BL23 and ATCC 334, and MaeKR expression was induced by L-malic acid [9]. The genome sequences of *L. casei* strains BL23 and ATCC 334 harbor 17 putative TCSs, among which the role of three TCSs involved in bile response, cell envelope stress response, oxidative stress tolerance, and acid tolerance [10]. However, the role of TCSs in *L. casei* is not still well understood.

With the advance of large scale sequencing technologies and bioinformatics tools, it has become possible to computationally predict TCS proteins and their putative functions from the whole genome of an organism. The availability of complete genome sequences of six *L. casei* strains enables a more comprehensive study of the role of TCS in the stress response of this organism. In this study, we conducted a thorough comparative analysis of the identified TCS proteins which provides valuable insights into the conservation and divergence of TCS proteins in the *L. casei* strains studied here.

Materials and Methods

Data Collection

Complete genome sequences of the *L. casei* strains ATCC 334, LC2W, BD-II, BL23, W56 and str. Zhang were collected from the National Center for Biotechnology Information (NCBI) (ftp.ncbi.nih.gov/genomes/Bacteria/). The genomes used in this study was listed in Table 1.

Identification of HKs and RRs

The approach used to identify putative HKs and RRs from the complete genome sequences of *L. casei* strains ATCC 334, LC2W, BD-II, BL23, W56 and str. Zhang was similar to that described previously [11]. Briefly, the HMM profile (Accession numbers PF00512) was found in Pfam database that targets the HisKA family of HKs, which was used to recognize the HKs in the *L. casei* genomes. A profile HMM downloaded from Pfam protein families database [12], which targets the RR REC domain (Accession number PF00072), was used to recognize the RRs in each *L. casei* genome. Recovered sequences were further scrutinized according to the following criteria: (i) the HATPase domain had to be located in the C-terminus (last 2/3) of the encoded protein and (ii) a putative H-box had to precede the HATPase domain. Detection of HK–RR gene pairs and 'orphan' HK and RR genes was similar to that described previously [11].

Identification of Common and Unique TCS Proteins

TCS proteins that are common or unique among *L. casei* strains were identified through ortholog analysis. The ortholog groups were constructed by using the OrthoMCL-DB tool (http://www.orthomcl.org) [13]. Briefly, HK protein sequences of *L. casei* strains were assigned to OrthoMCL-DB for the ortholog group identification, and HK proteins belong to the same orthomcl_group were recognized as a common TCS protein.

Bioinformatic Analysis

Protein domain organizations of the HKs and RRs were identified using SMART (smart.embl-heidelberg.de) [14]. Domain limits for proteins were also derived from the

Table 2 Identification of putative two component systems in the six sequenced *L. casei* strains

Strains	*L. casei* ATCC 334	*L. casei* BD-II	*L. casei* BL23	*L. casei* LC2W	*L. casei* str. Zhang	*L. casei* W56
Total TCS proteins	31	29	31	31	31	30
Total paired HKs	15	14	15	15	15	13
Orphan HKs	0	0	0	0	0	1
Total paired RRs	15	14	15	15	15	13
Orphan RRs	1	1	1	1	1	3

graphical output of the SMART web interface. Transmembrane helices of HKs were predicted by the TMHMM2 program (http://www.cbs.dtu.dk/services/TMHMM/) [15]. Phylogenetic trees of the HKs and RRs were built by the software MEGA version 4 [16].

PCR Verification

To verify the presence of 15 TCSs in *L. casei*, PCR amplification with original DNA from two *L. casei* strains ATCC334, LC2W and five isolated strains was performed. The primers were designed using the PRIMER-BLAST at online NCBI. Conditions for this conventional PCR were: 94 °C, 2 min; followed by 30 cycles of 94 °C for 30 s; annealing temperature 58 °C for 30 s; and 72 °C for 30 s; final extension at 72 °C for 5 min. The amplified PCR products were resolved in a 1.5 % agarose gel.

Results and Discussion

Identification of TCS Proteins of *L. casei* Strains

The putative HKs and RRs in the six *L. casei* strains were identified by searching the complete genome sequences for proteins containing HK and RR domains using Pfam HMM profiles. The repertoires of potential TCS proteins (HKs and RRs) were obtained, as shown in Table 2. By analyzing the putative operon organizations of genes encoding the identified TCS proteins 98.9 % of the total putative HKs and 93.1 % of the total putative RR were found to constitute HK–RR pairs. No hybrid HKs could be detected in all the genomes of the six *L. casei* strains compared in this study.

Ortholog Analysis of TCS Proteins

Ortholog analysis of the paired or non-paired TCS proteins among the six *L. casei* strains revealed a total of 15 different TCS clusters, one orphan HKs and three orphan RRs (Table 3). Co-evolution of TCS proteins could be clearly observed. This means, HKs and RRs which belong to a particular TCS cluster are usually co-present or co-absent in a specific strain. Twelve of the 15 TCS clusters (designated as TCS-2, TCS-4, TCS-5, TCS-7, TCS-8, TCS-9, TCS-10, TCS-11, TCS-12, TCS-13, TCS-14, TCS-15) were common to all the strains. Three clusters (TCS-1, TCS-3, TCS-6) were observed to be absent in one or several strains. One orphan HK was identified as uniquely present in *L. casei* W56 (BN19407810, named as orphan HK1). In contrast, an orphan RR (orphan RR1, LSEI2389 in *L. casei* ATCC 334) was found to be common to all strains except for *L. casei* BD-II. *L. casei* W56 harbored an additional unique orphan RR (BN19402120, orphan RR3). In addition, a clear clustering of HK and RR orthologs can be visualized in the phylogenetic tree shown in Fig. 1, which additionally illustrates the relationships between the different TCS clusters.

Classification of HKs Based on Domain Architecture Analysis

Using the classification method as previously described [17], the putative HKs were grouped into three different groups: extracytoplasmic-sensing HKs, cytoplasmic-sensing HKs, and membrane-sensing HKs (HKs with sensing mechanisms associated with membrane-spanning helices), as shown in Fig. 2.

Among all the HKs identified, HKs of TCS-3 and TCS-4 were recognized as extracytoplasmic sensing HKs by displaying at the N-terminal region an extracytoplasmic putative signal perception domain, which were flanked by (at least) two transmembrane helices (TMs). The cytoplasmic part of the HK proteins harboring the transmitter domain comprised either a HisKA-HATPase_c domain (HK of TCS-3) or a PAS-HisKA-HATPase_c domain (HK of TCS-4). Per-Arnt-Sim (PAS) domains play important roles as sensory modules for sensing oxygen tension, cellular redox state, or light intensity [18]. Most PAS domain-containing proteins are intracellularly located with dual functions of monitoring both the external and internal environments by perceiving alterations in the electron transport system caused by intracellular or extracellular changes in redox potential [19]. It should be noticed that a region of low compositional complexity was found to exist between two TM regions of the TCS-3 HK. The region starts at position 121 and ends at position 135.

Table 3 Ortholog analysis and classifications of the putative TCS proteins in the six sequenced *L. casei* strains

TCS cluster	TCS protein	Family	*L. casei* ATCC 334	*L. casei* BD-II	*L. casei* BL23	*L. casei* LC2W	*L. casei* str. Zhang	*L. casei* W56
TCS-1	HK	IIIA	LSEI0220	LCBD0209	LCABL02090	LC2W0200	LCAZH0244	Absent
	RR	OmpR	LSEI0219	LCBD0208	LCABL02080	LC2W0199	LCAZH0243	Absent
TCS-2	HK	IIIA	LSEI0461	LCBD0525	LCABL05270	LC2W0527	LCAZH0491	BN19405340
	RR	OmpR	LSEI0460	LCBD0524	LCABL05260	LC2W0526	LCAZH0490	BN19405330
TCS-3	HK	IIIA	LSEI0712	LCBD0787	LCABL07770	LC2W0786	LCAZH0650	Absent
	RR	OmpR	LSEI0711	LCBD0786	LCABL07760	LC2W0785	LCAZH0649	Absent
TCS-4	HK	IIIA	LSEI0935	LCBD1030	LCABL10490	LC2W1035	LCAZH0878	BN19410230
	RR	OmpR	LSEI0934	LCBD1029	LCABL10480	LC2W1034	LCAZH0877	BN19410220
TCS-5	HK	IIIA	LSEI0951	LCBD1046	LCABL10650	LC2W1051	LCAZH0894	BN19410390
	RR	OmpR	LSEI0950	LCBD1045	LCABL10640	LC2W1050	LCAZH0893	BN19410380
TCS-6	HK	IIIA	LSEI1042	Absent	LCABL12060	LC2W1201	LCAZH1021	BN19411800
	RR	OmpR	LSEI1041	Absent	LCABL12050	LC2W1200	LCAZH1020	BN19411790
TCS-7	HK	IIIA	LSEI1208	LCBD1406	LCABL14270	LC2W1374	LCAZH1199	BN19414030
	RR	OmpR	LSEI1209	LCBD1407	LCABL14280	LC2W1375	LCAZH1200	BN19414040
TCS-8	HK	II	LSEI1223	LCBD1422	LCABL14440	LC2W1390	LCAZH1215	BN19414190
	RR	CitB	LSEI1222	LCBD1421	LCABL14430	LC2W1389	LCAZH1214	BN19414180
TCS-9	HK	IIIA	LSEI1419	LCBD1621	LCABL16420	LC2W1589	LCAZH1407	BN19416160
	RR	OmpR	LSEI1420	LCBD1622	LCABL16430	LC2W1590	LCAZH1408	BN19416170
TCS-10	HK	II	LSEI1666	LCBD1862	LCABL18840	LC2W1841	LCAZH1656	BN19418500
	RR	CitB	LSEI1665	LCBD1861	LCABL18830	LC2W1840	LCAZH1655	BN19418490
TCS-11	HK	IIIA	LSEI1678	LCBD1874	LCABL18970	LC2W1853	LCAZH1668	BN19418640
	RR	OmpR	LSEI1679	LCBD1875	LCABL18980	LC2W1854	LCAZH1669	BN19418650
TCS-12	HK	IIIA	LSEI1741	LCBD1939	LCABL19610	LC2W1918	LCAZH1733	BN19419260
	RR	OmpR	LSEI1740	LCBD1938	LCABL19600	LC2W1917	LCAZH1732	BN19419250
TCS-13	HK	?	LSEI2600	LCBD2778	LCABL27660	LC2W2754	LCAZH2565	BN19427090
	RR	LytT	LSEI2599	LCBD2777	LCABL27650	LC2W2753	LCAZH2564	BN19427080
TCS-14	HK	IIIA	LSEI2680	LCBD2898	LCABL28710	LC2W2872	LCAZH2680	BN19428160
	RR	OmpR	LSEI2681	LCBD2899	LCABL28720	LC2W2873	LCAZH2681	BN19428170
TCS-15	HK	IIIA	LSEI2807	LCBD3032	LCABL30120	LC2W3017	LCAZH2819	BN19429500
	RR	OmpR	LSEI2808	LCBD3033	LCABL30130	LC2W3018	LCAZH2820	BN19429510
Orphan HK1	HK	IIIA	Absent	Absent	Absent	Absent	Absent	BN19407810
Orphan RR1	RR	LytT	LSEI2389	Absent	LCABL25620	LC2W2549	LCAZH2351	BN19425170
Orphan RR2	RR	OmpR	Absent	LCBD1189	Absent	Absent	Absent	BN19407790
Orphan RR3	RR	OmpR	Absent	Absent	Absent	Absent	Absent	BN19402120

The HKs of seven TCS clusters, namely the soluble HK of TCS-11 and the membrane anchored HKs of TCS-2, TCS-6, TCS-7, TCS-10, TCS-14, and TCS-15, and Orphan HK1 were identified as HKs with putative cytoplasmic sensing functions.

HKs of TCS-2, TCS-6, TCS-11, TCS-14, and TCS-15 possess a histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins and phosphatases (HAMP) domain. HAMP functions as a linker to bridge the transmembrane helix and the transmitter domain [20]. HK of TCS-15 possesses the PAS and PAC domains. It has been reported that PAS domains are often associated with proxy auto-config (PAC) domains and they are directly linked and together form the conserved 3D PAS fold [21], as also exemplified by the HK of TCS-15 in this study.

HKs of TCS-1, TCS-5, TCS-8, TCS-9, TCS-12 and TCS-13 in this study were all found to belong to membrane-sensing HK group, indicating that a relatively high

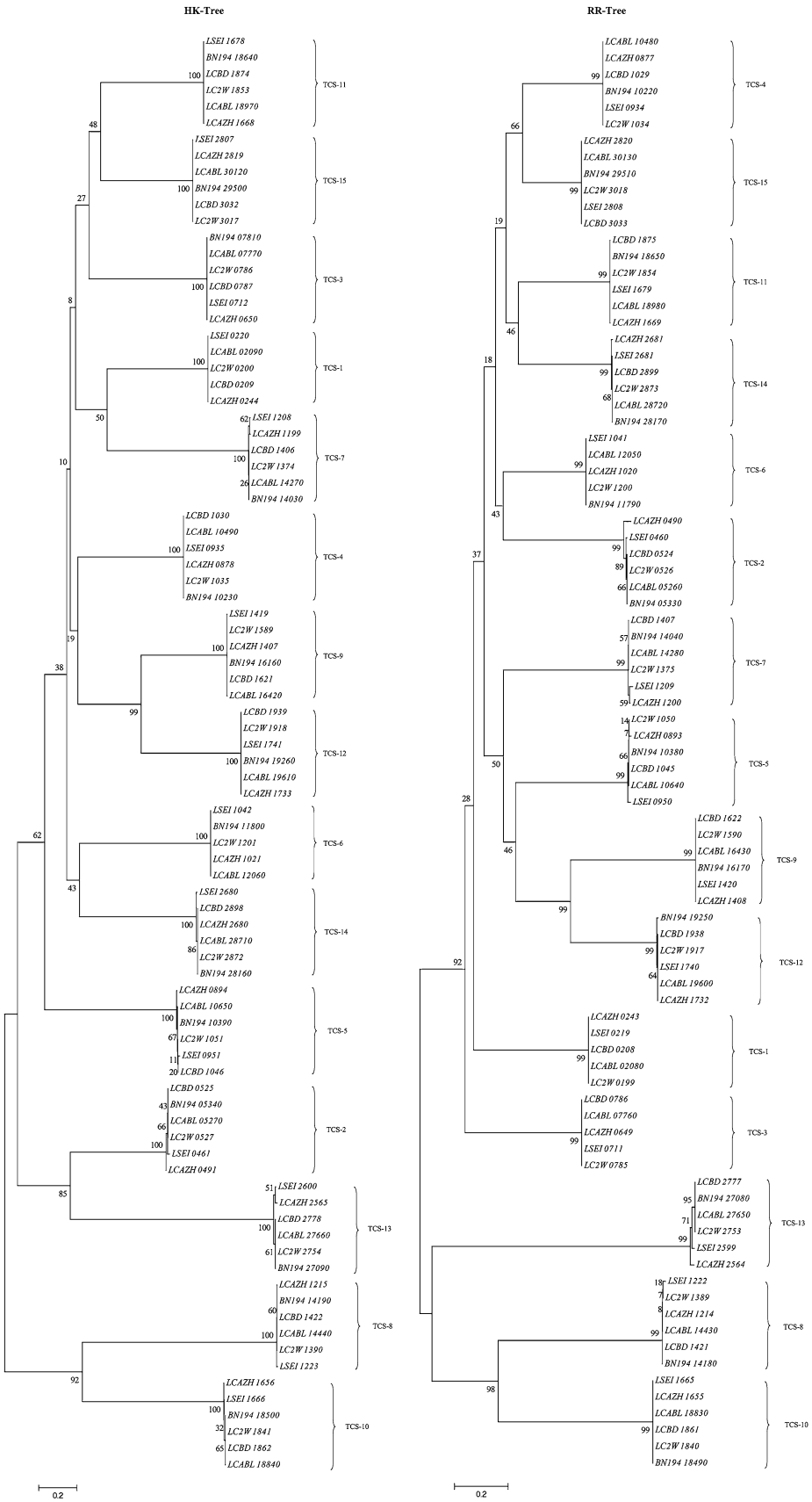

Fig. 1 Phylogenetic trees of the paired HKs and RRs in the six sequenced *L. casei* strains. The trees were constructed using MEGA version 4 by applying the neighbor-joining method. The *scale bar* is shown above the trees and the *scale* is in units of "substitution/site"

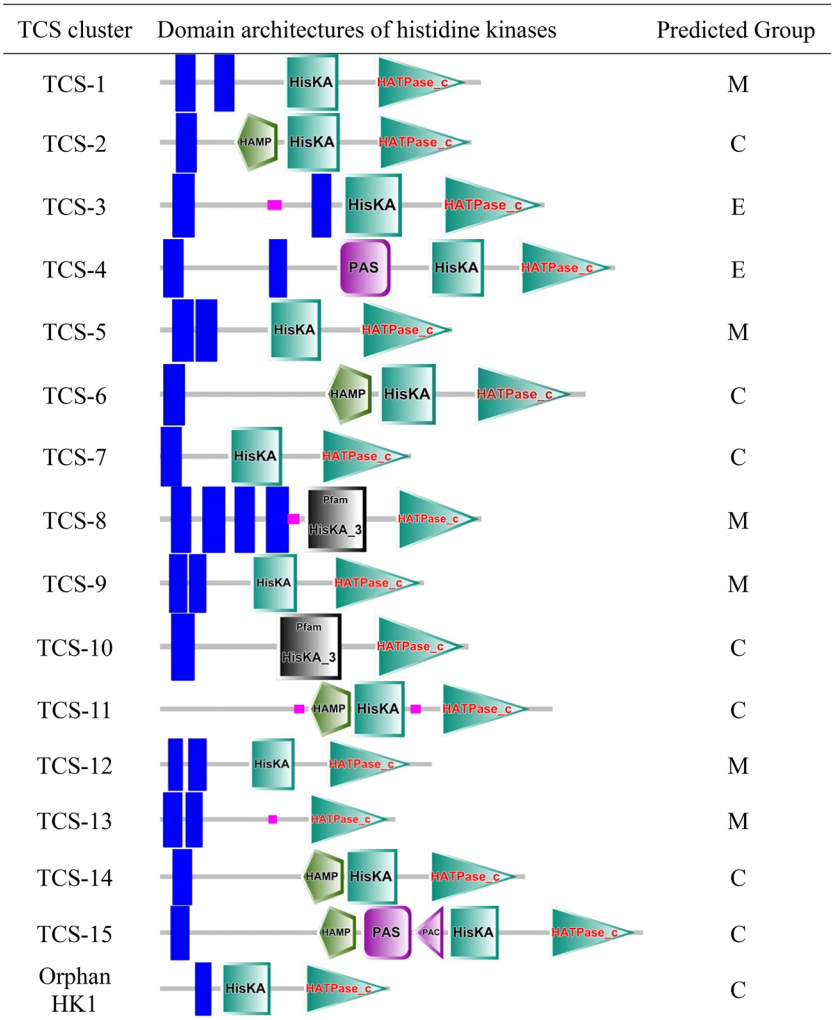

Fig. 2 Domain architectures of histidine kinases representative of each TCS clusters. The pictorial depiction is based on the predictions carried out using the SMART web interface http://smart.embl-heidelberg.de/. The transmembrane helices (TMs) were predicted using the tool TMHMM. *C*, *E* and *M* stand for cytoplasmic, extracytoplasmic and membrane sensing, respectively

percentage of HKs of the *L. casei* strains are involved in sensing signals directly associated with the membrane.

Classification of RRs

The majority of the putative RRs identified in this study were classified into the following 3 families: CitB, LytT and OmpR, with RRs of the OmpR family constituting the largest group. The assignment of RRs of the 15 TCS clusters and the orphan RRs to the corresponding RR families is given in Table 3.

The RRs of TCS-8 and TCS-10 contain a protein of the CitB family, respectively. Members of the CitB family have been documented to control expression of the genes for citrate fermentation in response to external citrate under anaerobic conditions [22, 23], and to have an effect on the inheritance of iteron-containing plasmids and on the SOS response to β-lactam antibiotics [24, 25].

The RR of TCS-13 and Orphan RR1 contain a protein of the LytT family, respectively. RR proteins of the LytT family are characterized by having a non-HTH DNA binding domain, which modulate the expression of many genes coding for virulence factors, fimbriae, cell wall components, bacteriocins, extracellular polysaccharides etc. [26, 27].

The RRs of the others 12 TCS clusters and two orphan RRs contain a protein of the OmpR family, respectively. RRs of the OmpR family constituted the largest group. Proteins of the OmpR family have been reported to mediate a wide range of biological functions related to, for example, osmolarity, phosphate assimilation, antibiotic resistance, virulence and toxicity [28].

TCS Proteins Common to All the Six *L. casei* Strains

TCSs are conserved in closely related microorganisms [29–31]. Ancient TCSs, on one hand, may have maintained basic functions in different bacteria, and on the other hand, may also have evolved new functionalities in niche-specific bacteria.

Table 4 A brief summary of known/putative functions of the TCSs identified in *L. casei* BL23

TCS cluster	TCS protein	GenBank locus tag	Functions	References
TCS-1	HK-hpk31	LCABL02090	Bile and NaCl response, antimicrobials response, cell envelope stress tolerance	[10]
	RR-rrp11	LCABL02080		
TCS-2	HK-resE	LCABL05270	Unclear	
	RR-spaR	LCABL05260		
TCS-3	HK-ciaH	LCABL07770	Unclear	
	RR-llrF	LCABL07760		
TCS-4	HK-hpk2	LCABL10490	Antimicrobials resistance	[10]
	RR-rrp2	LCABL10480		
TCS-5	HK-resE	LCABL10650	Unclear	
	RR	LCABL10640		
TCS-6	HK-hpk7	LCABL12060	Cell envelope stress response, nisin resistance, bile and NaCl response, oxidative stress tolerance, H_2O_2 stress tolerance	[10, 35–37]
	RR-rrp7	LCABL12050		
TCS-7	HK	LCABL14270	Unclear	
	RR	LCABL14280		
TCS-8	HK-hpk6	LCABL14440	Unclear	
	RR-rrp6	LCABL14430		
TCS-9	HK-hk07	LCABL16420	Cell envelope stress response, bacitracin and nisin resistance	[10, 32]
	RR	LCABL16430		
TCS-10	HK	LCABL18840	Antimicrobials response,	[10]
	RR	LCABL18830		
TCS-11	HK-hpk5	LCABL18970	Nisin resistance	[10]
	RR-rrp5	LCABL18980		
TCS-12	HK-hpk1	LCABL19610	Cell envelope stress response, acid tolerance, nisin resistance	[10, 32]
	RR-rrp1	LCABL19600		
TCS-13	HK	LCABL27660	Unclear	
	RR	LCABL27650		
TCS-14	HK-kinE	LCABL28710	Bacitracin resistance	[10]
	RR-rrp2	LCABL28720		
TCS-15	HK-hpk3	LCABL30120	Bacitracin resistance	[10]
	RR-rrp3	LCABL30130		
Orphan RR1	RR-pltR	LCABL25620	Unclear	

Proteins of the TCS clusters 2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15 are common to all the six *L. casei* strains compared here, indicating probably the functional importance of these TCS clusters for the adaptation and survival of these *L. casei* strains isolated almost from dairy products (Tables 1, 3, 4). For instance, TCS-12 is highly conserved across the six *L. casei* strains. TCS-12 is homologous to the three paralogous TCS of *Bacillus subtilis*, BceRS, YvcPQ, and YxdJK, involved in the cell envelope stress response against the nisin [10, 32]. TCS-12 of *L. casei* BL23 strain has been found to play a vital role in the growth under a low pH environment [10]. Therefore, it is conceivable that conservation of TCS-12 across the *L. casei* strains is essential for their acid tolerance. These functional similarities and differences of the core TCSs clearly indicate that although they are conserved in the *L. casei*, they may have developed new niche-specific functions during evolution.

L. casei strains have achieved popularity in the manufacture of probiotic products because of their convincing beneficial effects on human health. However, before providing benefit to human health, *L. casei* strains have to survive the difficult journey through the human gastrointestinal tract in sufficient densities in the presence of bile salts [33]. The implicated pathways of *L. casei* are involved with a complex physiological response under bile salts stress, particularly including cell protection (DnaK and GroEL), modifications in cell membranes (NagA, GalU, and PyrD), and key components of central metabolism (PFK, PGM, CysK, LuxS, PepC, and EF-Tu) [34].

In this study, we found that TCS-1 and TCS-6 clusters are involved with the response under bile salts stress, and TCS-12 is involved with the response under the acid tolerance. In addition, the TCS-6 cluster is also involved in the stress tolerance of oxidative and H_2O_2. These TCSs in *L. casei* will contribute to survive the difficult journey through the human gastrointestinal tract.

TCS Proteins Uniquely Present/Absent in One or Several Strains

The TCS-1 cluster was predicted to be absent in *L. casei* W56 strain, which is involved in cell envelope stress tolerance, and the response of the bile, NaCl and antimicrobials of *L. casei* BL23 (Table 4) [10]. The TCS-3 was also absent in *L. casei* W56. Taken together, these findings indicate dramatic differences in the regulation of the response of the bile, NaCl, antimicrobials and the cell envelope stress of *L. casei* W56 in comparison to the other *L. casei* strains.

The TCS-6 cluster could be not found in *L. casei* BD-II strain, which is involved in cell envelope stress response, nisin resistance, bile and NaCl response, oxidative stress tolerance, H_2O_2 stress tolerance of *L. casei* BL23 strain [10, 35–37]. Orphan RR1 was also absent in *L. casei* BL23 strain.

It has been suggested that specific TCS systems may play critical roles in microbe–host relationship, such as the HrpXY system in plant enterobacteria, which regulates type III secretion [38].

PCR Verification of Predicted TCSs

To verify the presence of predicted TCSs in *L. casei*, 15 primer pairs were designed based on 15 TCS genes, respectively (Supporting Information, Table S1). PCR amplifications using these primers were performed with two sequenced *L. casei* strains (*L. casei* ATCC334 and LC2W), four isolated strains (*L. casei* BD00054, BD00090, BD01649, and BD01803) and one *L. paracasei* BD03416. All primer sets exhibited 100 % inclusivity for six *L. casei* strains (Supporting Information, file 1). However, no clearly products were obtained from the isolated strain *L. paracasei* BD03416, which needs to be further clarified. Typical data is shown in Fig. 3. These results supported successfully the identification of these TCSs in *L. casei* strains by bioinformatics analysis.

Conclusion

In the present study we conducted a genome-wide identification, classification, and ortholog analysis of the TCS proteins in six sequenced *L. casei* strains. Totally, 15 TCS clusters comprising HK–RR pairs were identified, with 12 of them shared by all the six strains compared, three being absent in one strain. In addition, one orphan HKs and three orphan RRs were identified. We believe that the results from this genomic level study will be certainly helpful for the design of physiological studies which in turn will lead to a better understanding of response mechanisms for survival in the gastrointestinal tract of *L. casei* strains.

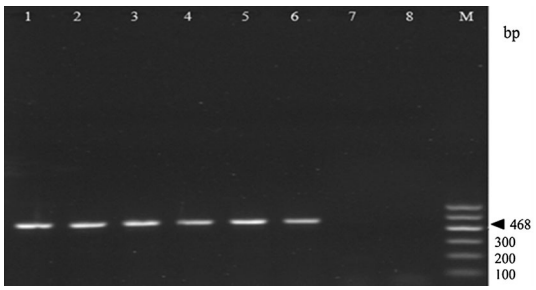

Fig. 3 PCR verification of the presence of TCS genes in *Lactobacillus casei* strains. Agarose gel electrophoresis of PCR products amplified using TCS-2 primers. *Lane 1*, *Lactobacillus casei* ATCC334; *Lane 2*, *Lactobacillus casei* LC2W; *Lane 3*, *Lactobacillus casei* BD00054; *Lane 4*, *Lactobacillus casei* BD00090; *Lane 5*, *Lactobacillus casei* BD01649; *Lane 6*, *Lactobacillus casei* BD01803; *Lane 7*, *Lactobacillus paracasei* BD03416; *Lane 8*, negative control (ddH_2O); *M* 100 bp DNA Marker

Acknowledgments This work was supported by the Open Project Program of State Key Laboratory of Dairy Biotechnology, Bright Dairy & Food Co. Ltd., (No. SKLDB2012-007).

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