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Complete genome sequence of *Lactococcus lactis* subsp. *lactis* KLDS4.0325, a bacterium newly isolated from Koumiss in Xinjiang, China

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Abstract: [**Objective**] Aim to study the physiology and functionally important genes of *Lactobacillus lactis* KLDS4.0325. [**Methods**] We sequenced the complete genome of *L. lactis* KLDS4.0325, drew the genomic map, and performed functional annotation and analysis in metabolism and probiotic. [**Results**] *L. lactis* KLDS4.0325 contains a chromosome of 2589261 bp, GC content is 35.4%, with 2662 predicted ORFs, of which 1310 are functionally classified. *L. lactis* KLDS4.0325 can carry out hydrolysis of extracellular proteins effectively, has the potential to degrade bitter peptides and produce a series of peptides of inhibiting angiotensin converting enzyme. *L. lactis* KLDS4.0325 has complete enzyme system for transamination pathway, which can catalyze relevant amino acids to flavor compounds. More key enzyme-coding genes involved in transport and metabolizing of sugars, and L-lactic acid synthesis, are exist in *L. lactis* KLDS4.0325 genome. *L. lactis* KLDS4.0325 has a set of more complete encoding genes in the biosynthetic pathways of folate and riboflavin. In addition, gene cluster for Lactococcin and 2 cold stress protein (*cspD* and *cspE*) were identified. [**Conclusion**] The presence of these genes encoding desirable traits provides the theoretical basis for the strain in industrial fermentation, and relevant further research.

Keywords: genome sequence proteolytic system, amino acid metabolism, B-Group vitamin biosynthesis, bacteriocin biosynthesis, cold stress protein biosynthesis, in silico analysis
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Lactococcus lactis is a mesophilic, Gram-positive bacterium, which can ferment hexose to lactic acid. They are widely used for the production of fermented food products, such as yogurt, cheese and others[1-8]. *L. lactis* strains are subdivided into two lineages, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, based on geno- and pheno-typing.

We report the complete genome sequence of *L.*

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lactis KLDS4.0325, a probiotic bacterium isolated from home-make koumiss in Xinjiang of China. In order to reveal the relationship between genetic content and their physiological functions, the whole genome shot gun strategy was used for genome sequencing of *L. lactis* KLDS4.0325, which was cultured by Key laboratory of Dairy Science, Ministry of Education, Northeast Agriculture University, China. After assembly and annotation, bioinformatics methods were used for analyzing *L. lactis* KLDS4.0325 genome. This work contributes to exploit the potential of *L. lactis* KLDS4.0325 as industrial fermentation strains, and provides direction for strain character improvement.

1 Materials and Methods

1.1 Genome Sequencing and Assembly

Genomic DNA from *L. lactis* KLDS4.0325 was used to construct a genomic library with the Stratagene Lambda Fix II _ *Xho*I Partial Fill-in Vector. The complete genome sequence of *L. lactis* KLDS4.0325 was determined by whole-genome shotgun sequencing, using Sanger technology, the insert sizes of clone library is 500 bp. The genome was assembled by using SOAPdenovo software, multiplex PCR was used to close the gaps, and remove low-covered regions.

1.2 Bioinformatics Analysis

Softwares Gamola and Glimmer were used for automatic annotation process and determining the gene model of the complete genome sequence, respectively. Against the non-redundant protein database from National Center for Biotechnology Information (NCBI) and UniProt Knowledgebase, the gapped BLASTP algorithm was performed to analyze sequence similarity. The clusters of orthologous proteins (COG) database (applied threshold 1e－10) was used for the functional classification. Using PFAM-HMM libraries, HMMER was used for determining protein motifs. With both the stringent and the relaxed parameter sets, TRNASCAN-SE was used to identify tRNAs. Nucleotide repeats were identified and visualized by the KODON software package. CGVIEW software was used to obtain visual genome atlas. Pathway reconstructions of *L. lactis* KLDS4.0325 were performed by using the KEGG (Kyoto Encyclopedia of Genes and Genomes) on-line database. The chromosome complete genome of *L. lactis* KLDS4.0325 has been deposited in GenBank, under accession number CP006766.

The complete genome sequences for other 9 reference strains were obtained from the NCBI microbial genome database. These genomes includes: *Lactococcus lactis* subsp. *lactis* IL1403 (abbreviation LLA, accession number AE005176), *Lactococcus lactis* subsp. *lactis* KF147 (LLK, CP001834), *Lactococcus lactis* subsp. *Lactis* CV56 (LLT, CP002365), *Lactococcus lactis* subsp. *lactis* IO-1 (LLS, AP012281), *Lactococcus lactis* subsp. *cremoris* SK11 (LLC, CP000425), *Lactococcus lactis* subsp. *cremoris* MG1363 (LLM, AM406671), *Lactococcus lactis* subsp. *cremoris* A76 (LLR, CP003132), *Lactococcus lactis* subsp. *cremoris* NZ9000 (LLN, CP002094), *Lactococcus lactis* subsp. *cremoris* UC509.9 (LLI, CP003157).

2 Results and Discussion

2.1 General Genome Features

The complete genome of *L. lactis* KLDS4.0325 contains a single, circular chromosome of 2589261 bp and three plasmids [plasmid1 (5.7 kb), plasmid2 (2.1 kb) and plasmid3 (2.7 kb)]. The overall G + C content of the chromosome is 35.4%, with 2662 predicted ORFs (Figure 1), of which 1310 were functionally classified. 62 tRNA encoding genes and 6 rRNA encoding genes were identified. The GC content of plasmids was 34.7%, with 212 predicted ORFs. Comparative analysis of the *L. lactis* KLDS4.0325 with 4 other *L. lactis* genomes were performed using Mummer, SplitTree4 and Mauve. Results show that *L. lactis* KLDS4.0325 shares 2438 ORFs with *L. lactis* IL1403, *L. lactis* CV56, *L. lactis* KF147, *and L. cremoris* NZ9000, and 2215 ORFs have 80% sequence identity[5]. Predicted ORFs of *L. lactis* KLDS4.0325 in COG database function classifies showed that the

majority of predicted proteins belong to 3 functional classes, those are transcription, replication, recombination and repair; carbohydrate transport and metabolism, amino acid transport and metabolism, and so on. Only 23% are assigned to the "poorly characterized".

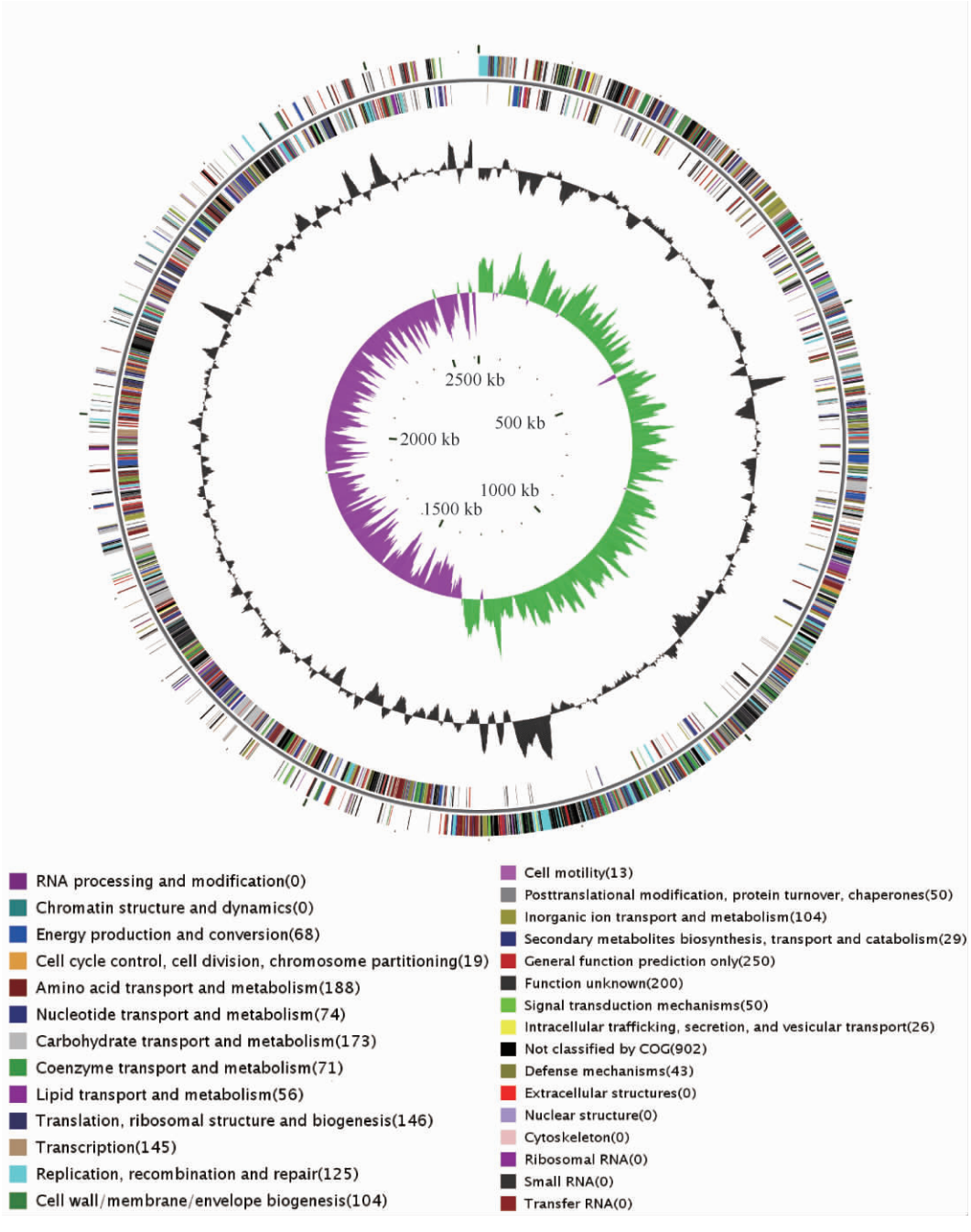

Figure 1. General features of *L. lactis* KLDS4.0325 genome. The circle was created by GCVIEW software, 6 circles from the outer to the inner. Outermost circle 1 shows the distribution of plus strand ncRNA, includes tRNA, rRNA, and sRNA; circle 2 shows the distribution of plus strand COG annotation genes, and distinguishes them in different colors; circle 3 shows the distribution of negative strand COG annotation genes; circle 4 shows the distribution of negative strand ncRNA; circle 5 (black) shows G + C content, which takes the average G + C content as a baseline, the part of outward show higher average, otherwise below average; circle 6 shows the value of G + C skew, purple represents that the value is less than 0, whereas green represents that the value is greater than 0.

2.2 Proteolytic System

Lactic acid bacteria （LAB） proteolytic system plays a key role in its growth, especially. The functions of cell-wall bound protenase （CEP） and peptidase are necessary for cell growth under conditions containing different types of nitrogen[9]. Gene deletion studies have shown that LAB are unable to grow in milk under the circumstance of the absence of a functional CEP[10]. Proteolysis of casein by LAB is initiated by a cell-wall bound protenase （PrtP）, which can degrade proteins into oligopeptides. The oligopeptides are subsequently taken up by peptide transport system, and further degraded into di/tri-peptides or amino acids by a set of intracellular peptidases[11-12].

For cell-wall bound proteinase, most of LAB possess only 1 CEP, but 2 were identified in the *L. lactis* KLDS4.0325 genome （Table 1）, which were PrtP and PrtM, respectively. At present, PrtP was only found in the genomes of *L. cremoris* SK11, *S. thermophilus* LMD9, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. johnsonii* and *L. rhamnosus*[13]. Further studies have reported that PrtM plays a vital role in automatic activation of PrtP. For peptide transport systems, *L. lactis* KLDS4.0325 possesses 3 LAB peptide transport systems. In the oligopeptide Opp transport system, includes OPPA （Copy number is 3）, OPPB （2）, OPPC （2）, OPPF （2）; Di-/tripeptide transport system, includes Dppc （1）; Di-/tripeptide ion-linked transporter, includes DtpT （1）[14]. Consequently, *L. lactis* KLDS4.0325 can transport the majority of peptides into the cell by different peptide transport systems. Up to now, *Lactobacillus acidophilus* NCFM, *Lactobacillus brevis* ATCC 367, *Lactobacillus helveticus* DPC 4571, *Lactobacillus rhamnosus* GG, *Lactococcus lactis* subsp. *lactis* IL1403, *Lactococcus lactis* subsp. *cremoris* MG1363 and *Lactococcus lactis* subsp. *cremoris* SK11 possess all three known LAB peptide transport systems. Therefore, these strains show significant capabilities in various peptides transport. For peptidase, in the *L. lactis* KLDS4.0325 genome, endopeptidase encoding genes PepO （2） and PepF （2） were identified, which contribute to degrade bitter peptides produced in fermentation process. Besides, we also obtained aminopeptidase encoding gene, such as PepC （2）, PepN （2） and PepM （1）, which can inhibit the activity of angiotensin converting enzyme by producing related bioactive peptide.

2.3 Flavour Formation by Amino Acid Catabolism

Enzymatic degradation of amino acids, in particular, the branched-chain amino acids, methionine and aromatic amino acids, are the important reason for the formation of key-flavour components in dairy fermentation, most notably cheeses[15].

Transaminases can catalyse amino acids conversion to its corresponding α-keto acid, which are converted to aldehydes, hydroxyl acids and CoA-esters. All of these can give the cheese a unique flavor and aroma. In *L. lactis* KLDS4.0325 genome, 1 branched-chain amino acid aminotransferase （BcAT） encoding gene was identified （Table 2）, which displays an activity towards both the branched-chain amino acids and methionine. So *L. lactis* KLDS4.0325 is rich in capacity to catalytic valine, leucine and isoleucine to form α-ketoisocaproate, α-keto-β-methyl valeric acid and α-ketoisovaleric acid （the flavour components in fermented sausage）, respectively. Besides, 1 aromatic aminotransferase （ArAT） encoding gene was also identified, which is active against aromatic amino acids, methionine and leucine. Therefore, *L. lactis* KLDS4.0325 can transform phenylalanine, tyrosine and tryptophan into phenylpyruvic acid, p-hydroxy pyruvic acid, and indole pyruvate, respectively. We also found 2 aspartate aminotransferase （AspAT） encoding genes in *L. lactis* KLDS4.0325 genome, which is responsible for aspartate transamination, but also active against the aromatic amino acids. It is believed that flavor compounds, such as 2-methylbutanoate （sweaty odor） and isobutyric acid （sour and sweet odor） are derived from the transamination of Ile or Val by BcAT in cheese. The encoding genes of α-keto acid dehydrogenase, phosphotransacylase and acyl kinase

were identified, which can transform α-keto branched chain amino acid into acetoacetic acid, α-methylbutyric acid and isobutyric acid, and all of them taste sweet. We also found the encoding gene of α-keto acid decarboxylase (KdcA), indicating that *L. lactis* KLDS4.0325 may carry out decarboxylation reaction against branched chain amino acid with α-keto, and produces the corresponding aldehyde. For example, it can convert α-ketoisocaproate, α-keto-β-methyl valeric acid and α-ketoisovaleric acid into isovaleraldehyde (grass flavor), activity valeraldehyde (malt fragrance) and isobutylaldehyde (banana flavor), respectively. Besides, 6 alcohol dehydrogenase genes and 2 aldehyde dehydrogenase genes were identified in *L. lactis* KLDS4.0325 genome and their copy numbers are more than other common strains. Therefore, *L. lactis* KLDS4.0325 may be more effective to catalyze aldehyde into corresponding carboxylic acids and alcohols, such as the fresh cheese isoamylol and sweaty 2-Methylbutanoic acid, and so on. Esters with short-chain fatty acids are important for the development of cheeses with the characteristic fruity flavor. A gene named *xynC*, which can encode an acetylesterase for catalyzing the biosynthesis of esters (fruity flavor). Such as ethyl butanoate, ethyl caproate, and ethyl-3-methylbutanoate, etc. Therefore, *L. lactis* KLDS4.0325 fermented dairy products may produce some esters for short-chain fatty acids.

Table 1. Distribution of proteinase, peptide transporters and peptidase of the proteolytic system in *L. lactis* KLDS4.0325

proteolytic system	peptidase	family	substrate/annotation	KLDS4.0325
proteinase				
cell-wall bound proteinase	PrpP	S8-A		1[p]
	PrtM		maturation protein for PrtP	1[p]
peptides transporters				
oligopeptides ABC transport system	OPPA		oligopeptide-binding protein	3
	OPPB		permease protein	2
	OPPC		permease protein	2
	OPPD		ATP-binding protein	0
	OPPF		ATP-binding protein	2
di/tripeptides ABC transport system	DppA/P		di/tripeptide-oligopeptide-binding protein	0
	DppB		permease protein	0
	DppC		permease protein	1
	DppD		ATP-binding protein	0
	DppF		ATP-binding protein	0
di/tripeptides ion-linked transporter	DtpT		PTR family	1
Peptidases				
aminopeptidase	PepC	C1-B	Xl(X)n	1, 1[p]
	PepN	M1	Xl(X)n	1, 1[p]
unique aminopeptidases	PepM	M24-A	metl(X)n	1[p]
	PepA	M1	glu/aspl(X)n	1,1[p]
	Pcp	C15	pyro/glul(X)n	
endopeptidase	PepE/PepG	C1-B	(X)ml(X)n	0
	PepO	M13	(X)ml(X)n	1,1[p]
	PepF	M3-B	(X)ml(X)n	2
dripeptidase	PepD	C69	XlX	2,1[p]
	PepV	M20-A	XlX	1,1[p]
tripeptidase	PepT	M20-B	XlX-X	1,1[p]
proline peptidase	PepX	S15	X-Prol(X)n	1,1[p]
	Pepl	S33	prolX-(X)n	1
	PepR	S33	prolX	1
PepL	S33	leul(X)n	0	
	PepP	M24-B	XlPro-(X)n	1[p]
	PepQ	M24-B	XlPro	1

[p] represents the number of encoding gene exists in plasmid genome of *L. lactis* KLDS4.0325.

Table 2. Distribution of the enzymes involved in transamination pathways of amino acid degradation in *L. lactis* KLDS4. 0325

orf _id	abbreviation	gene name	functional description
klds4_orf01430	BcAT	*bcaT*	branched-chain aminotransferase
klds4_orf00032	ArAT	*araT*	aromatatic aminotransferase
klds4_orf02047	AspAT	*aspB*	aspartate aminotransferase
klds4_orf00208	AspAT	*aspC*	aspartate aminotransferase
klds4_orf01470	KdcA	*kdcA*	alpha-ketoisovalerate decarboxylase
klds4_orf00268	AlcDH	*lwe*1062	alcohol dehydrogenase
klds4_orf01662	AlcDH	*LLNZ*_04975	oxidoreductase
klds4_orf01669	AlcDH	*ypjA*	zinc-binding alcohol dehydrogenase
klds4_orf02023	AlcDH	*adhA*	alcohol dehydrogenase
klds4_orf00752	AlcDH	*MW*2112	zinc-type alcohol dehydrogenase-like protein
klds4_orf00289	AlcDH	*SPBC*16*A*3. 02*c*	zinc-type alcohol dehydrogenase-like protein
klds4_orf01806	Bifunctional AldDH/AlcDHa	*asd*	aspartate-semialdehyde dehydrogenase
klds4_orf02492	Bifunctional AldDH/AlcDHa	*adhE*	alcohol-acetaldehyde dehydrogenase
klds4_orf00035	KaDH complex	*KadA/B/C/D*	keto acid dehydrogenase complex
klds4_orf01843	PTA	*pta*	(branched chain) phosphotransacylase
klds4_orf02308	ACK	*ackA*	acetate kinase
klds4_orf02307	ACK	*ackA*	acetate kinase
klds4_orf02261	D-HycDH	*yugC*	d-isomer specific 2-hydroxyacid dehydrogenase
klds4_orf01983	EstA	*xynC*	acetyl esterase

2.4 Sugar Metabolism

Lactate fermentation by lactic acid bacteria is considered as the most important fermentation process employed in food technology[16]. To exploit the potential of sugar metabolism of *L. lactis* KLDS4. 0325, bioinformatics methods were used to analysis transport of extracellular saccharides, metabolism and acid production. The results showed that the *L. lactis* KLDS4. 0325 genome possesses more key enzyme encoding genes those involved in transport, metabolism for sugar, and L-lactic acid production.

Genes for 9 phosphotransferase system (PTS) protein complexes were found in the *L. lactis* KLDS4. 0325 genome, those include mannitol, fructose, mannose, sucrose, glucoside, cellobiose, lactose, and permease genes of gluconate, lactose and galactose (Table 3). The number of encoding genes of PTS protein in *L. lactis* KLDS4. 0325 genome are more abundant than that in other *Lactococcus lactis* suggests the flexibility of *L. lactis* KLDS4. 0325 in sugars utilization (Table 4). The sugars will be converted into intermediate products of glycolysis after transported inside cells with a series of enzymes catalyst encoding by *L. lactis* KLDS4. 0325 genome (Table 5), such as the conversion of D-Mannitol 1-phosphate to beta-D-Fructose 6-phosphate. *L. lactis* KLDS4. 0325 possesses perfect complete enzyme system for L-lactic acid biosynthetic pathway. During the conversion of alpha-D-Glucose 1-phosphate to alpha-D-Glucose 6-phosphate, the key enzyme encoding gene of phosphoglucomutase exists only in *L. lactis* KLDS4. 0325 genome, but not found in other 9 reference strains (Table 6). Meantime, in the process of D-glycerate 1, 3-diphosphate is converted to 3-phosphoglycerate, bisphosphoglycerate and bisphosphoglycerate mutase, two additional enzyme encoding genes in this process, were not existed in 9 reference strains, but were identified in *L. lactis* KLDS4. 0325 genome. Therefore, *L. lactis* KLDS4. 0325 can take advantage of more substrates to produce lactic acid compared with the reference strains. Above all, in *L. lactis* KLDS4. 0325 genome, 5 L-lactate dehydrogenase encoding genes were identified, which is far more than 9 reference strains. However, we didn't found any encoding gene relates to D-lactate dehydrogenase or lactate racemase in *L. lactis* KLDS4. 0325 genome. Therefore, *L. lactis* KLDS4. 0325 has the ability to utilize various sugars to produce L-lactic acid, but not D-lactic acid. *L. lactis* KLDS4. 0325 may be a probiotics of high yield L-lactic acid, with industrial potential.

Table 3. PTS related protein encoding genes in the *L. lactis* KLDS4. 0325 genome

orf_id	description
klds4_orf00024	PTS system, mannitol-specific IIBC component
klds4_orf00026	PTS system , mannitol-specific IIA component
klds4_orf00225	PTS system, cellobiose-specific transporter subunit IIC
klds4_orf00506	PTS system, cellobiose-specific IIB component
klds4_orf00507	PTS system, cellobiose-specific IIA component
klds4_orf00509	PTS system, cellobiose-specific transporter subunit IIC
klds4_orf00860	PTS system, cellobiose-specific IIC component
klds4_orf01198	PTS system, sucrose-specific transporter subunit IIABC
klds4_orf00520	PTS system, sucrose-specific EIIBCA component
klds4_orf00521	PTS system, beta-glucoside-specific IIABC component
klds4_orf01574	PTS system, beta-glucoside-specific transporter subunit
klds4_orf01071	PTS system, fructose-specific EIIABC component IIABC
klds4_orf01895	mannose-specific PTS system component IIAB
klds4_orf01896	mannose-specific PTS system component IIC
klds4_orf01897	mannose-specific PTS system component IID
scaffold2_orf00062	PTS system lactose-specific EIICB component
scaffold2_orf00061	lactose-specific phosphotransferase enzyme IIA component

Table 4. Distribution of sugar transporters in the genomes of 10 strains *Lactococcus lactis*

sugar	transporter	KLDS	LLA	LLK	LLT	LLS	LLM	LLN	LLC	LLR	LLI
mannitol	phosphotransferase system (PTS) gene complex	1	0	1	1	1	1	1	0	1	1
fructose		1	0	0	0	0	0	0	1	0	1
mannose		1	1	0	0	1	0	0	0	1	1
sucrose		2	0	0	2	0	0	0	0	0	0
lactose		1	0	0	0	0	0	0	0	0	0
galactitol	permease	0	0	0	0	0	0	0	0	0	0
gluconate		1	0	1	1	0	0	0	0	0	0
maltose		0	0	0	0	0	0	0	0	0	1
lactose		1	0	1	1	0	0	0	0	0	0
galactose		1	0	0	1	0	1	1	0	1	1

KLDS, llA, LLK, LLT, LLS, LLM, LLN, LLC, LLR and LLI represent *Lactococcus lactis* subsp. *lactis* KLDS4. 0325, *Lactococcus lactis* subsp. *lactis* IL1403, *Lactococcus lactis* subsp. *lactis* KF147, *Lactococcus lactis* subsp. *lactis* CV56, *Lactococcus lactis* subsp. *lactis* IO-1, *Lactococcus lactis* subsp. *cremoris* MG1363, *Lactococcus lactis* subsp. *cremoris* NZ9000, *Lactococcus lactis* subsp. *cremoris* SK11, *Lactococcus lactis* subsp. *cremoris* A76, *Lactococcus lactis* subsp. *cremoris* UC509. 9, respectively.

Table 5. Genes distribution of enzyme involving in the process of converted sugars into intermediate products of glycolysis after they were transported into cell in the genomes of 10 strains *Lactococcus lactis*

enzyme	KLDS	LLA	LLK	LLT	LLS	LLM	LLN	LLC	LLR	LLI
mannitol-1-phosphate 5-dehydrogenase [EC:1. 1. 1. 17]	1	1	1	1	1	1	1	1	1	1
1-phosphofructokinase [EC:2. 7. 1. 56] /tagatose-6-phosphate kinase	1	1	1	1	1	1	1	1	0	1
fructose-bisphosphate aldolase, class I [EC:4. 1. 2. 13]	1	1	1	1	1	1	1	1	1	1
fructose-bisphosphate aldolase, class I [EC:4. 1. 2. 13]	1	1	1	1	1	1	1	1	1	1
triosephosphate isomerase (TIM) [EC:5. 3. 1. 1]	1	1	1	1	1	1	1	1	1	1
triose kinase [EC 2. 7. 1. 28]	0	0	0	0	0	0	0	0	0	0
mannose-6-phosphate isomerase [EC:5. 3. 1. 8]	1	1	1	1	1	1	1	1	1	1
beta-fructofuranosidase /sucrase-6-phosphate hydrolase [EC:3. 2. 1. 26]	1	0	1	1	1	0	0	0	0	0
6-phospho-beta-galactosidase/phospho-beta-galactosidase [3. 2. 1. 85]	1	0	0	0	0	0	0	3	1	1
gluconokinase /gluconate kinase [EC:2. 7. 1. 12]	1	1	1	1	1	1	1	1	1	1
6-phosphogluconate dehydrogenase [EC:1. 1. 1. 44]	2	2	2	2	2	2	1	2	2	2
ribulose-phosphate 3-epimerase [EC:5. 1. 3. 1]	1	1	1	1	1	2	2	2	1	1
transketolase [EC:2. 2. 1. 1]	1	1	1	1		1	1	1	1	1
maltose phosphorylase [EC:2. 4. 1. 8]	1	1	1	1	2	2	1	1	1	1
beta-galactosidase [EC:3. 2. 1. 23]	1	1	2	1	0	0	0	0	0	0
galactokinase [EC:2. 7. 1. 6]	1	1	2	1	1	1	1	1	1	1
UDPglucose—hexose-1-phosphate uridylyltransferase [EC:2. 7. 7. 12]	2	1	2	2	1	2	2	1	2	2

Figures represents the copy number of coding gene of each enzyme.

Table 6. Distribution of genes for enzymes involving in L-lactate biosynthetic pathway in the genomes of 10 strains *Lactococcus lactis*

enzymes	KLDS	LLA	LLK	LLT	LLS	LLM	LLN	LLC	LLR	LLI
Phosphoglucomutase, [EC:5. 4. 2. 2]	1	0	0	0	0	0	0	0	0	0
glucose-1-phosphate phosphodismutase, [EC:2. 7. 1. 4] /glucose-1-phosphatase, [EC:3. 1. 3. 10]	0	0	0	0	0	0	0	0	0	0
hexokinase, [EC: 2. 7. 1. 1] /glucokinase, [EC: 2. 7. 1. 2] /polyphosphate glucokinase, [EC:2. 7. 1. 63] /ADP-dependent glucokinase, [EC:2. 7. 1. 147]	1	1	1	1	1	1	1	1	1	1
Aldose1-epimerase [EC:5. 1. 3. 3]	3	2	3	4	2	1	1	1	1	3
hexokinase [EC: 2. 7. 1. 1] /glucokinase, [EC: 2. 7. 1. 2] /polyphosphate glucokinase, [EC:2. 7. 1. 63] /ADP-dependent glucokinase, [EC:2. 7. 1. 147]	1	1	2	1	1	1	1	1	1	1
glucose-6-phosphate 1-epimerase, [EC: 5. 1. 3. 15] /glucose-6-phosphate isomerase, [EC:5. 3. 1. 9]	1	1	1	1	1	1	1	1	1	1
fructose-1,6-bisphosphatase, [EC:3. 1. 3. 11] /6-phosphofructokinase, [EC:2. 7. 1. 11] /ADP-dependent phosphofructokinase, [EC:2. 7. 1. 146]	2	2	2	2	2	2	2	2	2	2
fructose-bisphosphate aldolase, [EC:4. 1. 2. 13]	1	1	1	1	2	1	1	1	1	1
triosephosphate isomerase (TIM), [EC:5. 3. 1. 1]	1	1	1	1	1	1	1	1	1	1
glyceraldehyde 3-phosphate dehydrogenase, [EC:1. 2. 1. 12] /glyceraldehyde-3-phosphate dehydrogenase (NAD(P)), [EC:1. 2. 1. 59]	2	2	2	2	2	2	2	2	2	1
bisphosphoglycerate mutase, [5. 4. 2. 4]	1	0	0	0	0	0	0	0	0-	0
2,3-diphosphoglycerate phosphatase, [3. 1. 3. 13]	1	0	0	0	0	0	0	0	0-	0
Phosphoglycerate kinase, [EC:2. 7. 2. 3]	1	1	1	1	1	1	1	1	1	1
aldehyde: ferredoxin oxidoreductase, [EC: 1. 2. 7. 5] /glyceraldehyde-3-phosphate dehydrogenase (ferredoxin), [EC1. 2. 7. 6] /glyceraldehyde-3-phosphate dehydrogenase (NADP), [EC:1. 2. 1. 9]	0	0	0	0	0	0	0	0	0	0
phosphoglycerate mutase, [EC:5. 4. 2. 11]	4	3	3	1	4	4	4	3	3	3
phosphopyruvate hydratase/enolase, [4. 2. 1. 11]	2	1	2	2	2	2	2	2	2	2
pyruvate kinase, [EC:2. 7. 1. 40]	1	1	2	1	1	1	1	1	1	1
L-lactate dehydrogenase, [EC:1. 1. 1. 27]	5	3	3	3	3	3	1	4	4	1

Figures represents the copy number of coding gene of each enzyme.

2.5 B-Group vitamins

It is well known that humans lack the biosynthetic capacity for most vitamins, which must get through food or supplements. Fermented dairy products, produced by LAB-promoted biosynthesis, which has high-levels of B-group vitamins, such as folate and riboflavin, etc[17-18].

L. lactis KLDS4. 0325 possesses 2 folate biosynthetic pathways, which take guanosine 5′-triphosphate and chorismate as the substrate, respectively, through a series of enzyme-catalyzed, eventually synthetic folate. For the first way (Table 7), *L. lactis* KLDS4. 0325 has perfect complete enzyme systems encoding genes, especially it ecoding 2 alkaline phosphatase genes in the genome. which is a single copy gene, however, in the majority of 9 reference strains genome. Furthermore, the encoding genes of folylpolyglutamate synthase and dihydrofolate synthase are coexisted simultaneously in *L. lactis* KLDS4. 0325 genome, but the latter is not coexisted in the 9 reference strains genome. On the other hand, we also obtained a complete enzyme coding genes of folate biosynthetic pathways in *L. lactis* KLDS4. 0325 genome, but there are remarkable differences in the distribution of enzymes involving in folate biosynthetic pathway of 10 strains *Lactococcus lactis* genomes. For example, para-aminobenzoate synthetase is the first enzyme in the folate biosynthetic pathways, It can take chorismate as the substrate to produce 4-Amino-4-deoxychorismate, the copy number of the enzyme is 2 in *L. lactis* KLDS4. 0325, LLK, LLM, LLR, LLN and LLI genome, but it is 1 in other strains. *L. lactis* KLDS4. 0325 and 9 reference strains possess only 1 riboflavin biosynthetic pathway, and all the enzymes in

this pathway are the single copy gene (Table 8). For other B-group vitamins, such as VB_1, VB_3, VB_5, VB_6, VB_7 and VB_{12}, because of lacking key enzyme genes in these vitamins biosynthetic pathways in *L. lactis* KLDS4.0325 genome, which is not possible to synthesize them (Table 9).

Table 7. Distribution of genes for the enzymes involving in folate biosynthetic pathway in the genomes of 10 *Lactococcus lactis*

enzyme	KLDS	LLA	LLK	LLT	LLS	LLC	LLM	LLR	LLN	LLI
GTP cyclohydrolase I, [EC:3.5.4.16], *folE*	1	0	0	0	0	1	1	0	0	0
alkaline phosphatase, [EC:3.1.3.1], *phoA/ phoB*	2	1	0	0	0	1	2	0	0	0
[EC:3.6.1.-]	0	0	0	0	0	0	0	0	0	0
[EC:3.6.1.-]	0	0	0	0	0	0	0	0	0	0
dihydroneopterin aldolase, [EC:4.1.2.25], *folB*	1	1	1	1	1	1	1	1	1	1
2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase, [EC: 2.7.6.3], *folK*	1	1	1	0	0	1	1	0	0	0
7,8-dihydropteroate synthetase, [EC: 2.5.1.15], *folP*	1	1	1	1	1	1	1	1	1	1
para-aminobenzoate synthetase, [EC: 2.6.1.85], *pabAB*	2	1	2	2	2	1	1	2	2	1
4-amino-4-deoxychorismate lyase, [EC: 4.1.3.38], *pabC*	1	1	1	1	1	1	1	1	1	1
7,8-dihydropteroate synthase, [EC: 2.5.1.15], *folP*	1	1	1	1	1	1	1	1	1	1
dihydrofolate synthase/folylpolyglutamate synthase, [EC: 6.3.2.12 6.3.2.17], *folc*	2	1	2	1	1	1	2	2	2	2
dihydrofolate reductase, [EC:1.5.1.3], *folA*	1	1	1	1	1	1	1	1	1	1

Figures represents the copy number of coding gene of each enzyme.

Table 8. Distribution of genes for the enzymes involving in riboflavin biosynthetic pathway in the genomes of 10 strains *Lactococcus lactis*

enzyme	KLDS	LLA	LLK	LLT	LLS	LLC	LLM	LLR	LLN	LLI
GTP cyclohydrolase II, [EC: 3.5.4.25]	1	1	2	1	1	1	1	1	1	1
GTP cyclohydrolase IIa, [EC: 3.5.4.29]	0	0	0	0	0	0	0	0	0	0
2-amino-5-formylamino-6-ribosylaminopyrimidin-4(3H)-one 5′-monophosphate deformylase, [EC: 3.5.1.102]	0	0	0	0	0	0	0	0	0	0
diaminohydroxyphosphoribosylaminopyrimidine deaminase, [EC: 3.5.4.26]	1	1	1	1	1	0	1	2	1	1
5-amino-6-(5-phosphoribosylamino) uracil reductase, [EC: 1.1.1.193]	1	1	1	1	1	0	1	2	1	1
2, 5-diamino-6-(ribosylamino)-4 (3H)-pyrimidinone 5′-phosphate reductase, [EC: 1.1.1.302]	0	0	0	0	0	0	0	0	0	0
RIB2, PUS8, [EC:5.4.99.-]	0	0	0	0	0	0	0	0	0	0
[EC:3.1.3.-]	0	0	0	0	0	0	0	0	0	0
3,4-dihydroxy 2-butanone 4-phosphate synthase, [EC: 4.1.99.12]	1	1	1	1	1	1	1	1	1	1
6,7-dimethyl-8-ribityllumazine synthase, [EC: 2.5.1.78]	1	1	1	1	1	1	1	1	1	1
riboflavin synthase, [EC: 2.5.1.9]	1	1	1	1	1	1	1	1	1	1

Figures represents the copy number of coding gene of each enzyme.

Table 9. Absence of the genes for the key enzymes in the pathways of VB_1, VB_3, VB_5, VB_6, VB_7 and VB_{12} biosynthesis in the genome of *L. lactis* KLDS4.0325

vitamins	missed key enzymes
VB_1 (Thiamine metabolism)	Thiamin monophosphate phosphohydrolase, [EC:3.1.3.-]
VB_3 (Nicotinate and nicotinamide metabolism)	Nicotinate-nucleotide pyrophosphorylase, [EC: 2.4.2.19]
VB_5 (Pantothenate and CoA biosynthesis)	Pantetheine hydrolase, [EC: 3.5.1.92], Pantoate-beta-alanine ligase, [EC: 6.3.2.1] and pantothenase, [EC: 3.5.1.22]
VB_6 (Pyridoxine)	Erythronate-4-phosphate dehydrogenase, [EC: 1.1.1.290], Pyridoxal phosphatase, [EC: 3.1.3.74]
VB_7 (Biotin metabolism)	Biotinidase, [EC: 3.5.1.12], Biotin synthase, [EC: 2.8.1.6]
VB_{12} (Cyanocobalamin)	It is unclear what are the encoding genes of key enzymes for the VB_{12} biosynthetic pathways in lactic acid bacteria

2.6 Bacteriocin

Bacteriocins are the well-known antimicrobial compounds isolated from LAB. They are irreproducible peptides and exhibit bactericidal activity against phylogenetic close relatives[19-22]. In the genome of *L. lactis* KLDS4.0325, one gene cluster of Lactococcin was identified, which locates at 163227-79849 nt and contains 24 putative genes. The genetic organization of the locus was a typical of lactococcin (Table 10), including various structural genes, which plays a major role in bacteriocins precursor synthesis (such as lactococcin-B precursor). The lactococcin-B immunity protein can promote the hosts to develop immunity against the bacteriocin. Lactococcin A ABC transporter ATP-binding protein is mainly used for bacteriocin transport.

Table 10. The genes for bacteriocins gene cluster in the genome of *L. lactis* KLDS4.0325

table of genes	locations(bp)	strands	annotations
ctg1_orf00068	62,181 -63,351	+	glyoxalase family protein
ctg1_orf00069	63,361 -63,952	+	nitrilotriacetate monooxygenase
ctg1_orf00071	64,129 - 64,246	+	Hypoxanthine phosphoribosyltransferase
ctg1_orf00072	64,498 - 66,697	+	lactococcin A ABC transporter ATP-binding protein/permease
ctg1_orf00073	66,711 -67,950	+	lactococcin A secretion protein
ctg1_orf00074	68,226 -68,364	+	lactococcin-B precursor
ctg1_orf00075	68,378 -69,089	+	hypothetical protein
ctg1_orf00076	69,598 -69,967	+	transporter
ctg1_orf00078	71,148 -71,328	-	hypothetical protein
ctg1_orf00079	71,348 -71,528	-	lactococcin A1 precursor
ctg1_allorf000847	71,789 -71,899	+	lactococcin-B precursor
ctg1_orf00080	71,910 -72,198	+	lactococcin-B immunity protein
ctg1_orf00081	72,430 -72,613	+	hypothetical protein
ctg1_orf00082	72,614 -72,869	+	hypothetical protein
ctg1_orf00083	72,919 -74,158	+	hypothetical protein
ctg1_orf00084	74,377 -74,680	-	hypothetical protein
ctg1_orf00085	74,681 -74,849	-	hypothetical protein
ctg1_orf00086	75,045 -75,171	+	hypothetical protein
ctg1_orf00088	75,542 -75,698	-	phage integrase family protein
ctg1_orf00089	76,895 -77,060	-	hypothetical protein
ctg1_orf00090	77,196 -78,228	-	plasmid replication protein Rep
ctg1_orf00091	78,788 -79,052	-	hypothetical protein
ctg1_orf00093	79,148 -79,478	-	hypothetical protein
ctg1_orf00094	79,749 -80,781	-	ftsK/apoIIIE like protein

2.7 Cold shock proteins

1Bacteria can adapt to the temperatures far below the optimal temperature, and produce a series of 7-kDa proteins in response to a sharp decreased temperature[23]. Insight into the LAB psychrophilic contributors to overcome the occurrence of post acidification during yoghurt preservation and provides the basis for the living animal maintain a certain cell number after increasing the freezing temperature[24].

Through sequence similarity alignment, cold shock proteins *cspD* and *cspE* were identified in *L. lactis* KLDS4.0325 genome. Among them, the amino acid sequence of *cspD* of *L. lactis* KLDS4.0325 exhibited 100% identity with *Lactococcus lactis* subsp. *lactis* IL1403. Under certain circumstances, such as entering into stationary phase and carbon starving, *cspD* is induced. The express of *cspD* is mainly regulated by (p) ppGppp level, but not dependent on σ^s[25]. After the function analysis was performed for CspD of *Lactococcus lactis* subsp. *cremoris* MG1363, Wouters et al.[26] found that the strain cells will specifically overproduce CspD when the temperature is around 30℃, and freeze survival rates has increased 2 - 10 times. This suggests that the 7 kDa CspD may improve

survivability after the strains were frozen. The amino acid sequence of *cspE L. lactis* KLDS4.0325 also exhibited 100% identity with *Lactococcus lactis* subsp. *lactis* IO-1. CspE is constitutively produced throughout the growth stages except the lag period and many cell functions owing to CspE. For example, it will be lead to resistance to camphor and chromosome condensation when over produces CspE. Furthermore, CspE is involving in regulating the expression of *rpoS* and *uspA*, which play an important role in regulation of carbon-metabolizing genes and coordinate glucose and acetate metabolism, respectively. In short, it is probably fair to say that in the complex stress response network of the cell, CspD and CspE tend to act as regulatory elements for the expression of the stress proteins[27].

In silico analysis of the sequenced complete genome of *L. lactis* KLDS4.0325, a series of strain-specific genes and gene clusters that associated with probiotic and fermentation properties were identified, therefore, we revealed a large number of significant characteristics for *L. lactis* KLDS4.0325, such as high hydrolysis efficiency to extracelluar protein, excellent ability to flavour formation by amino acid catabolism, It may be makes better use of multiple sugar to produce lactic acid, and synthesize folate and riboflavin, the lactococcin, and cold shock proteins *CspD* and *CspE* efficiently. The current study not only provides a new way for ones to investigate the fermentation properties of *L. lactis* KLDS4.0325, but also makes us more convinced that *L. lactis* KLDS4.0325 is one lactic acid bacteria with high industrial application value and extensive application prospect.

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一株从中国新疆地区的酸马奶中分离出的新菌株，乳酸乳球菌乳酸亚种 KLDS4.0325 的全基因组序列

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摘要：【目的】为了阐明乳酸乳球菌乳酸亚种 KLDS4.0325 的生理及代谢机制，并对其重要功能基因进行挖掘，我们对菌株 KLDS4.0325 的全基因组序列进行测定和基因组图谱的绘制，并利用生物软件和数据库完成对序列的注释及相关功能性分析。【方法】在菌株序列完成测序、组装和注释后，根据序列信息进行全基因组图谱的绘制，并对菌株的蛋白质水解系统、氨基酸来源的风味形成途径和 B-族维生素合成途径进行了比较基因组分析，并对细菌素合成基因组和冷应激蛋白基因进行了预测。【结果】菌株 KLDS4.0325 基因组全长 2589250 bp，G + C 含量为 35.4%，共预测出 2662 个开放阅读框，其中 1310 个具有潜在的生物学功能。菌株可以有效的对细胞外蛋白质进行有效的水解，具有潜在的降低苦味肽以及产生一系列能够抑制血管紧张素转化酶活性的活性肽。在转氨途径方面，菌株 KLDS4.0325 具有较为完成的酶系统，可以催化相关氨基酸转化为风味物质。在菌株 KLDS4.0325 的基因组中，我们发现了较多编码糖转运、代谢以及 L-乳酸合成的基因。关于叶酸和核黄素合成途径的编码基因在菌株 KLDS4.0325 的基因组中也较为完整。此外，我们在菌株 KLDS4.0325 的基因组中预测出了一个关于乳球菌素的基因簇和两个冷应激蛋白 CspD 和 cspE 的编码基因。【结论】这些编码菌株显著特性基因的存在为菌株 KLDS4.0325 能够进行工业发酵提供了理论基础，并为其进一步研究提供了方向。

关键词：蛋白质水解系统，氨基酸代谢，B-族维生素合成，细菌素基因簇，冷应激蛋白基因，生物信息学分析

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