Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Comparative genomic analysis of *Lactobacillus plantarum* ZJ316 reveals its genetic adaptation and potential probiotic profiles*#

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Received Apr. 20, 2016; Revision accepted May 24, 2016; Crosschecked July 13, 2016

Abstract: Objective: In previous studies, *Lactobacillus plantarum* ZJ316 showed probiotic properties, such as antimicrobial activity against various pathogens and the capacity to significantly improve pig growth and pork quality. The purpose of this study was to reveal the genes potentially related to its genetic adaptation and probiotic profiles based on comparative genomic analysis. Methods: The genome sequence of *L. plantarum* ZJ316 was compared with those of eight *L. plantarum* strains deposited in GenBank. BLASTN, Mauve, and MUMmer programs were used for genome alignment and comparison. CRISPRFinder was applied for searching the clustered regularly interspaced short palindromic repeats (CRISPRs). Results: We identified genes that encode proteins related to genetic adaptation and probiotic profiles, including carbohydrate transport and metabolism, proteolytic enzyme systems and amino acid biosynthesis, CRISPR adaptive immunity, stress responses, bile salt resistance, ability to adhere to the host intestinal wall, exopolysaccharide (EPS) biosynthesis, and bacteriocin biosynthesis. Conclusions: Comparative characterization of the *L. plantarum* ZJ316 genome provided the genetic basis for further elucidating the functional mechanisms of its probiotic properties. ZJ316 could be considered a potential probiotic candidate.

Key words: *Lactobacillus plantarum* ZJ316, Comparative genomics, Probiotics, Adaptation
http://dx.doi.org/10.1631/jzus.B1600176 **CLC number:** Q93

1 Introduction

Lactic acid bacteria (LAB) species, including lactobacilli and bifidobacteria, are relatively abundant inhabitants of the gastrointestinal tract (GIT) of humans and animals. These bacteria are generally regarded as safe (GRAS) and are currently used as probiotics (Presti *et al.*, 2015). The FAO/WHO (Food and Agriculture Organization of the United Nations/ World Health Organization) has defined a probiotic as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill *et al.*, 2014). In general, probiotics should be capable of dealing with stressful conditions (including in vitro environmental stresses and in vivo human GIT conditions such as acidic pH and bile salts), and have antimicrobial activity against potential pathogens, the ability to reduce pathogen adhesion and adhere to human epithelial cells.

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* Project supported by the International Science & Technology Cooperation Program of China (No. 2013DFA32330), the National Natural Science Foundation of China (Nos. 31540044 and 31271821), the National High-Tech R & D Program (863) of China (No. 2014AA022210-08), and the Natural Science Foundation of Zhejiang Province (No. LY16C200002), China

Electronic supplementary materials: The online version of this article (http://dx.doi.org/10.1631/jzus.B1600176) contains supplementary materials, which are available to authorized users

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Many LAB species, in conjunction with other members of the intestinal microbiota, are believed to contribute to host nutrition, intestinal pH, cell proliferation and differentiation, the immune system, and its innate and acquired responses to pathogens. These perceived health benefits of LAB, which are attributed to the production of antimicrobial metabolites such as bacteriocin, inhibitory enzymes, and organic acids, have driven the commercial exploitation of LAB species as active components of many functional foods and therapeutic adjuncts (Fernandez *et al.*, 2003; Citar *et al.*, 2015).

Lactobacillus plantarum is one member of the LAB probiotic species that can be isolated from a large variety of environmental niches, including human saliva, grass silage, kimchi, pickled cabbage, and cheese (Siezen and van Hylckama Vlieg, 2011). Some strains, such as ST-III (Wang *et al.*, 2011), have been used as starter cultures or probiotics in the food industry. *L. plantarum* ZJ316 was originally isolated from fecal samples of healthy infants and showed many probiotic properties such as significantly improving pig growth and pork quality, and antimicrobial activity against various pathogens in vitro including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* (Suo *et al.*, 2012). However, knowledge of the molecular mechanisms responsible for its probiotic properties is still limited.

Comparative genomic analysis from multiple species or strains can provide insights into the functional and evolutionary processes of genomes. In the present study, we aimed to reveal the genes that might be related to genetic adaptation and probiotic profiles of ZJ316 based on comparative genomic analysis. The whole genome sequences of ZJ316, which have been sequenced by our lab (Li *et al.*, 2013), and other *L. plantarum* strains such as WCFS1 (Siezen *et al.*, 2012), JDM1 (Zhang *et al.*, 2009), ST-III (Wang *et al.*, 2011) and NC8 (Axelsson *et al.*, 2012) allowed us to annotate the genome of ZJ316 and make further investigations. Analysis of the predicted genes, together with comparisons to the genomes of other *L. plantarum* strains, revealed that this bacterium has undergone specific genetic adaptations to colonize and survive in the intestinal tract, and encodes various probiotic related genes.

2 Materials and methods

2.1 Genome sequences of *L. plantarum* strains

Whole genome sequences of *L. plantarum* ZJ316 have been reported by our lab previously (Li *et al.*, 2013), and are deposited in GenBank under accession number CP004082. The sequences and annotations of another eight *L. plantarum* strains studied here were obtained from the NCBI (http://www.ncbi.nlm.nih.gov): *L. plantarum* WCFS1 (NC_004567), *L. plantarum* JDM1 (NC_012984), *L. plantarum* subsp. *plantarum* ST-III (NC_014554), *L. plantarum* subsp. *plantarum* P-8 (NC_021224), *L. plantarum* 16 (NC_021514), *L. plantarum* subsp. *plantarum* ATCC 14917 (NZ_ACGZ00000000), *L. plantarum* subsp. *plantarum* NC8 (AGRI00000000), and *L. plantarum* UCMA 3037 (APHP00000000).

2.2 Bioinformatic analyses

Whole genome comparisons of the *Lactobacillus* strains were performed using BLASTN and Mauve alignments (Siezen *et al.*, 2010). Genome synteny comparisons were performed using the set of genomes mentioned above. Whole genomes were compared at the nucleotide level using the MUMmer programs with default parameters (Kurtz *et al.*, 2004) and were viewed using the Artemis comparison tool (ACT) (Carver *et al.*, 2008). Clustered regularly interspaced short palindromic repeats (CRISPRs) were analyzed using CRISPRFinder (Grissa *et al.*, 2007).

2.3 Phylogenetic tree construction

A phylogenetic tree was constructed based on genome context networks as described by Ding *et al*. (2008). Briefly, we constructed the gene context networks from 19 *Lactobacillus* strains and determined the phylogeny based on the pair-wise similarity of these 19 genomes. The phylogenetic tree was constructed using TreeView.

3 Results and discussion

3.1 General genomic characteristics of *L. plantarum* ZJ316

A phylogenetic tree shows the phylogenetic relationships between ZJ316 and other strains from the

genus *Lactobacillus* (Fig. 1). This organism formed a distinct branch with *L. plantarum* strains, and *L. plantarum* IPLA88 was found to be the closest evolutionary relative of strain ZJ316. A total of 3159 genes were predicted as being protein coding sequences (CDSs, about 85% of the genome), with an average length of 858 bp. The remaining intergenic regions had an average length of 144 bp. Note that this average length of intergenic regions is shorter than that of other known *L. plantarum* genomes (164 bp), indicating that *L. plantarum* ZJ316 has a more compact genome. Among the predicted CDSs, 2563 proteins of *L. plantarum* ZJ316 were functionally categorized, and the proportions in each category were compared with those of other *L. plantarum* genomes (Table 1 and Fig. 2) (Tatusov *et al.*, 2000). Among all the *L. plantarum* strains, *L. plantarum* ZJ316 has the most genes that function in defense mechanisms [V], cell wall/membrane/envelope biogenesis, outer membrane [M], amino acid transport and metabolism [E], lipid transport and metabolism [I], inorganic ion transport and metabolism [P], and secondary metabolite biosynthesis, transport and catabolism [Q]. On the other hand, it has the fewest genes involved in signal transduction mechanisms [T]. Such an extensive genetic adaptation to material transport and metabolism is shared, to a high degree, with enteric *L. plantarum* and likely represents a specific genetic adaptation of bacteria residing in the GIT.

L. plantarum ZJ316 has three plasmids, pLP-ZJ101, pLP-ZJ102, and pLP-ZJ103, encoding 117 genes. A number of plasmids from LAB have been shown to encode multiple important phenotypic traits. Similarly, pLP-ZJ101 is predicted possibly to encode calcium-transporting ATPase (pZJ101_03), relaxase (pZJ101_07), and integrase (pZJ101_15). Plasmid pLP-ZJ102 encodes oligo-1, 6-glucosidase (pZJ102_27), and polysaccharide biosynthesis proteins (pZJ102_48, pZJ102_49, and pZJ102_50). Plasmid pLP-ZJ103 encodes the potassium transport system protein Kup1 (pZJ103_07), the manganese transport protein MntH (pZJ103_22), as well as the glycine/betaine/carnitine ABC transporters ProX, ProW, and ProV (pZJ103_31–pZJ103_33), which were also found previously in pST-III (Chen *et al.*, 2012). These proteins are thought to be involved in a multi-component binding-protein-dependent transport system for glycine, betaine, and carnitine, allowing accumulation of these compounds to high levels inside the cell in response to increased external osmolarity, which is related to adaptation to the GIT environment.

3.2 Central carbon metabolism and lifestyle adaptations

L. plantarum is a versatile and flexible organism and is able to grow on a wide variety of sugar sources. This phenotypic trait is reflected by the high

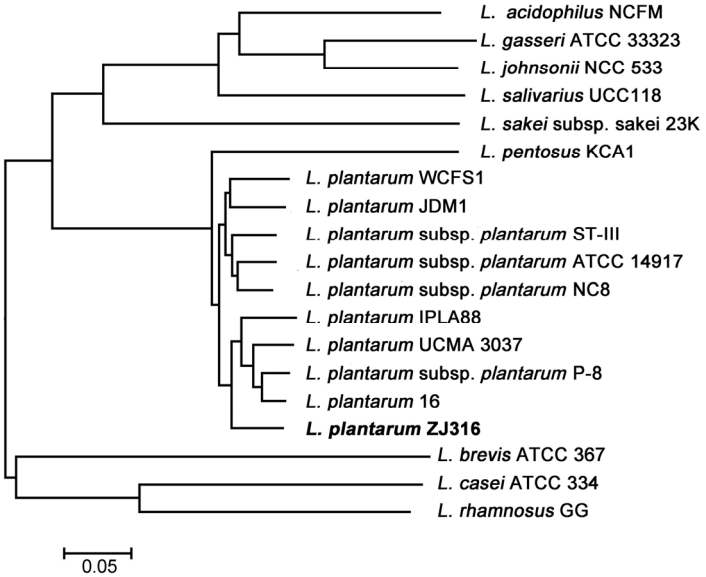

Fig. 1 Phylogenetic tree of ZJ316 and other *Lactobacillus* strains

Gene context networks were constructed from 19 *Lactobacillus* strains, and the homologs of all the strains were identified through gapped BLASTP

Table 1 Clusters of orthologous group (COG) functional categories in 10 completely sequenced genomes of *L. plantarum* strains

COG code	Strain									
	ZJ316	WCFS1	ST-III	JDM1	P-8	16	ATCC 14917	NC8	UCMA 3037	IPLA88
[J]	150	155	152	152	152	149	153	153	153	154
[A]	0	0	0	0	0	0	0	0	0	0
[K]	290	305	301	294	265	270	292	292	275	284
[L]	170	157	137	136	190	158	121	121	168	177
[B]	0	0	0	0	0	0	0	0	0	0
[D]	27	26	26	24	24	26	26	26	27	28
[Y]	0	0	0	0	0	0	0	0	0	0
[V]	63	60	60	60	60	62	56	56	61	59
[T]	88	100	95	100	89	93	94	94	95	99
[M]	160	156	153	142	129	130	152	152	140	146
[N]	7	11	7	9	7	8	8	8	6	6
[Z]	0	0	0	0	0	0	0	0	0	0
[W]	0	0	0	0	0	0	0	0	0	0
[U]	23	26	24	25	23	21	25	25	21	25
[O]	62	62	62	64	61	61	61	61	61	62
[C]	112	114	107	112	103	106	106	106	107	106
[G]	278	313	289	305	242	248	284	284	247	248
[E]	267	267	250	259	243	247	251	251	252	251
[F]	85	87	81	85	84	85	86	86	92	85
[H]	78	91	77	93	77	77	78	78	80	76
[I]	77	75	73	72	69	71	70	70	70	72
[P]	171	169	157	167	148	159	163	163	152	164
[Q]	42	42	38	38	37	36	40	40	37	38
[R]	413	421	394	404	369	382	405	405	381	390
[S]	220	224	220	223	212	209	221	221	215	222
No related COG	180	175	168	159	157	152	174	174	153	203
No hits	596	437	499	419	497	338	386	386	484	573
Total	3159	3058	2996	2948	2893	2778	2868	2868	2932	3116

[J] translation, [A] RNA processing and modification, [K] transcription, [L] replication, recombination and repair, [B] chromatin structure and dynamics, [D] cell cycle control, mitosis and meiosis, [Y] nuclear structure, [V] defense mechanisms, [T] signal transduction mechanisms, [M] cell wall/membrane biogenesis, [N] cell motility, [Z] cytoskeleton, [W] extracellular structures, [U] intracellular trafficking and secretion, [O] posttranslational modification, protein turnover, chaperones, [C] energy production and conversion, [G] carbohydrate transport and metabolism, [E] amino acid transport and metabolism, [F] nucleotide transport and metabolism, [H] coenzyme transport and metabolism, [I] lipid transport and metabolism, [P] inorganic ion transport and metabolism, [Q] secondary metabolites biosynthesis, transport and catabolism, [R] general function prediction only, [S] function unknown

number of genes encoding putative sugar transporters. The majority of these transporters are predicted phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase systems (PTSs). *L. plantarum* ZJ316 encodes 17 complete PTS enzyme II complexes and several incomplete complexes (Table S1). Once internalized, sugars are used as carbon sources for growth and for the generation of energy through fermentation. *L. plantarum* is usually grouped with the facultative heterofermentative lactobacilli, and sugars can be fermented via the Embden-Meyerhof-Parnas (EMP) pathway or the phosphoketolase pathway, leading to homolactic or heterolactic fermentation profiles, respectively (Kandler, 1983). In support of this classification, genes encoding enzymes involved in the EMP pathway were found in ZJ316.

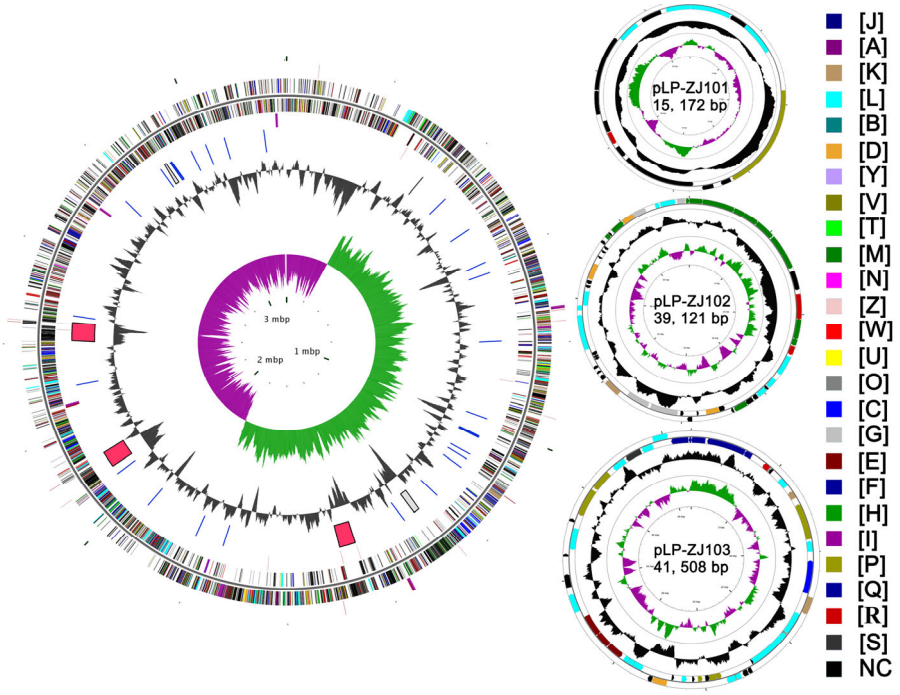

Fig. 2 Circular genome map of *L. plantarum* ZJ316

From the innermost circles, circle (1) illustrates the GC skew ((G−C)/(G+C)), where values >0 are in green, and values <0 are in purple. Circle (2) highlights the (G+C)% deviation from the mean (44.65%). Circle (3) denotes intact prophages in red, incomplete prophages in grey, and CRISPR repeats in black. Circle (4) indicates rRNAs (depicted in purple) and tRNAs (depicted in red) in the lagging replication strand. Coding regions are indicated by strand, and the color corresponds to the COG functional assignments; the lagging replication strand is shown in circle (5) and the leading replication strand in circle (6). Circle (7) denotes rRNA and tRNA genes in the leading replication strand (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

However, ZJ316 and ST-III lack both xylulose-5-P phosphoketolase and deoxyribose-phosphate aldolase, which prevent the conversion of D-xylulose 5-phosphate to D-glyceraldehyde 3-phosphate and the conversion between 2-deoxy-D-ribose 5-phosphate and D-glyceraldehyde 3-phosphate. As expected, the *L. plantarum* chromosome does not encode an intact citrate acid cycle. Moreover, the *ara*, *rha* and *iol* gene clusters responsible for arabinose, rhamnose, and *myo*-inositol utilization are not present in ZJ316, which indicates that the range of carbohydrate metabolism is constrained.

In particular, the 129 kb region from 92 000 to 221 000 almost exclusively encodes proteins for sugar transport, metabolism, and regulation. This region is much shorter than the 213 kb region in the other *L. plantarum* strains (Fig. 3). Moreover, this entire region has a lower G+C content (41.41%) than the rest of the genome (Fig. 2), suggesting that many genes may have been acquired through horizontal gene transfer (HGT). This finding supports the hypothesis that this part of the *L. plantarum* chromosome represents a lifestyle-adaptation region that is used to adapt effectively to changes in conditions encountered in certain environmental niches in which this microbe is found.

3.3 Proteolytic enzyme systems and amino acid biosynthesis

LAB generally inhabit protein-rich environments and are equipped with protein-degradation machinery to create a selective advantage for growth under these conditions. All *L. plantarum* genomes appear to encode the primary enzyme membrane-associated lipoprotein PrtM for the primary breakdown of proteins and large polypeptide utilization (Haandrikman *et al.*, 1991). The presence of *prtM* genes in both ZJ316 (zj316_1494 and zj316_3030) and other *L. plantarum* strains suggests that the organisms can digest large proteins extracellularly and

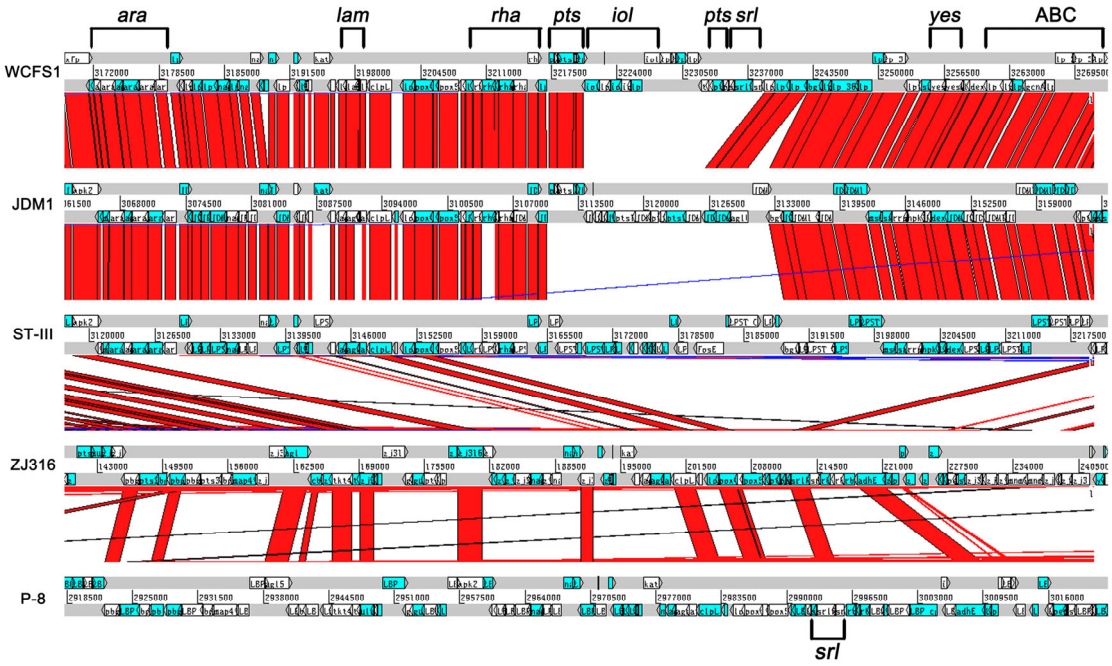

Fig. 3 Diversity of lifestyle adaptation region genes in *L. plantarum* strains

Comparison of genome organization surrounding the large cluster of lifestyle adaptation region genes. Genes are represented by blue and white arrows in the forward and reverse strands. The red connecting bars indicate high sequence identity and the blue bars a reverse orientation. The consecutive gene clusters, including arabinose biosynthesis (*ara*), auto-inducing peptide (*lam*), rhamnose biosynthesis (*rha*), PTS system (*pts*), *myo*-inositol biosynthesis (*iol*), sorbitol operon (*srl*), two-component (*yes*), and ABC transporter (ABC), are indicated in WCFS1 (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

generate primary protein-degradation products that are internalized by uptake systems (Opp and Dtp clusters). Two separated Opp clusters (zj316_1286–zj316_1290 and zj316_3100–zj316_3104), which are di- and oligo-peptide transporting systems, were predicted. This prediction is in contrast to the single cluster found in the other *L. plantarum* strains. As in other *L. plantarum* strains, only one permease-type transporter for di- and tri-peptides (*dtp*T, zj316_0697) was located in ZJ316. Scattered throughout the genome, two additional genes coding for distinct periplasmatic components (OppA) were identified and likely broaden the Opp-transporter specificities (zj316_0304 and zj316_0801).

Once internalized, these peptides are degraded by a variety of peptidases and proteases, which have been extensively studied in LAB (Savijoki *et al.*, 2006). ZJ316 has 34 genes encoding intracellular peptidases and 23 genes encoding proteases of different specificity (Table S2). Like other *L. plantarum* strains, ZJ316 encodes the complete biosynthetic pathways of most amino acids except valine, leucine, and isoleucine.

3.4 Stress response

Despite the presence of stress response machinery in all eight *L. plantarum* strains, special proteins such as the ImpB/MucB/SamB family proteins and proteins of nitrate/sulfonate/bicarbonate ABC transporter (zj316_0670 and zj316_0671) were identified in ZJ316. The nitrate/sulfonate/bicarbonate ABC transporter, which also can be found in strain ST-III, is up-regulated in response to salt-stress (Huang *et al.*, 2006). It may increase the efficiency of inorganic salt uptake and give the bacteria a way to survive and compete in the gut niche (Lurie-Weinberger *et al.*, 2012). Two genes, zj316_2874 and zj316_2875, code the ImpB/MucB/SamB family proteins, which are involved in ultraviolet (UV) protection. zj316_0075 and zj316_2984 encode two DNA-binding ferritin-like proteins (Dps family proteins). Both the ImpB/MucB/SamB and Dps family proteins play central roles in protecting DNA from oxidative damage by directly binding to DNA (Zhao *et al.*, 2002; Chang *et al.*, 2006).

3.5 Bile salt hydrolase

Bile is one of the most serious obstacles to bacterial survival in a mammalian gut. Conjugated bile acids (CBAs) have been reported to influence the intestinal microflora through direct antimicrobial effects, upregulation of host mucosal defenses, or synergistic action of both (Begley *et al.*, 2006; Kumar *et al.*, 2012). To survive in a gut, lactobacilli produce bile salt hydrolases (BSH) to deconjugate the amino acid moiety from CBAs. BSH activity has been detected and *bsh* genes identified in *Lactobacillus*, especially in the strains associated with the intestinal tract. Interestingly, two *bsh* genes (zj316_0150 and zj316_0281) were detected in the feces-derived strain ZJ316. The feature of multiple BSH homologs is also found in WCFS1, which has four *bsh* genes (Bron *et al.*, 2006). Begley *et al.* (2006) speculated that the multiple BSH feature may help the bacteria to tolerate different types of bile or perhaps promote bile adaptation, thus ensuring maximal survival of the bacteria under changing environmental conditions. ZJ316 might show good bile tolerance based on these genomic features.

3.6 Adhesion ability

Adhesion of bacteria to mucosal surfaces is necessary both to colonize the small intestinal tract and deliver the health benefits; thus adhesion ability is an important selection criterion for probiotics (Lavilla-Lerma *et al.*, 2013). Adhesion capacity might be associated with certain surface proteins, fatty acids, and exopolysaccharides (EPS). EF-Tu is a novel identified surface protein that has the characteristics of an adhesion factor and is capable of inducing a proinflammatory response. It functions as an adhesion-like factor in *Bifidobacterium* (Yuan *et al.*, 2008). EF-Tu was encoded by gene zj316_2121 in ZJ316, suggesting potential adhesion of the strain in the GIT.

3.7 Capsular polysaccharide (CPS) biosynthesis genes

EPS produced by LAB have received increasing attention because of their capacity to improve the textural properties of fermented dairy and non-dairy products (Yang *et al.*, 2010). CPS in LAB is the EPS attached at the bacterial surface and has been shown to be important for the colonization, adhesion, stress resistance, host-bacteria interactions, as well as immunomodulation of *Lactobacillus* strains. Recent evidence also shows that the *cps* clusters encoding polysaccharides contribute to the *L. plantarum* WCFS1 cell surface architecture and are probably related to TLR2 signaling (Remus *et al.*, 2012). Three *cps* clusters (*cps*3, *cps*4, and parts of the *cps*2 clusters) were identified in ZJ316, encoding proteins involved in biosynthesis and the export of CPSs (Fig. 4). Strain ST-III shares most of the *cps* clusters with ZJ316. However, strains JDM1, UCMA 3037, P8, and 16 have only the *cps*4 cluster. Strain WCFS1 has four *cps* clusters (*cps*1, *cps*2, *cps*3, and *cps*4) and the largest number of *cps* genes (Remus *et al.*, 2012). It has been reported that many probiotic properties of ST-III, including cholesterol removal, may relate to CPSs (Wang *et al.*, 2011). We speculate that the presence of *cps* clusters in ZJ316 might be of benefit for its probiotic properties and gut adaptation.

3.8 Plantaricin biosynthesis genes

Bacteriocins are antimicrobial peptides produced by bacteria that inhibit the growth of similar or closely related bacterial strains, and have been considered as potential drug candidates for replacing antibiotics (Alvarez-Sieiro *et al.*, 2016). Bacteriocins produced by *L. plantarum* strains facilitate their competitive ability in the environment and maximize their survival chances. The plantaricin (*pln*) locus is responsible for bacteriocin biosynthesis in *L. plantarum*. The *pln* gene cluster of *L. plantarum* contains about 25 genes (Fig. 5) and encodes various class II bacteriocins (Diep *et al.*, 2009; Nissen-Meyer *et al.*, 2010). It was found to be a highly variable and mosaic region, with some parts relatively conserved and other parts less conserved (Diep *et al.*, 2009; Saenz *et al.*, 2009). The *pln* loci of nine fully sequenced *L. plantarum* strains were also highly variable (Fig. 5). Twenty-two genes related to class II bacteriocins were identified in the genome of ZJ316. ZJ316 has genes encoding the two-peptide plantaricin PlnJK and the inducible class IIb plantaricin NC8βα, and the corresponding immunity protein NC8c (Maldonado *et al.*, 2003). ZJ316 and NC8 share the regulatory operon, which includes the inducing peptide PLNC8IF and the histidine protein kinase PLNC8K (Maldonado *et al.*, 2004), while the other strains use a different regulatory operon, *pln*ABCD.

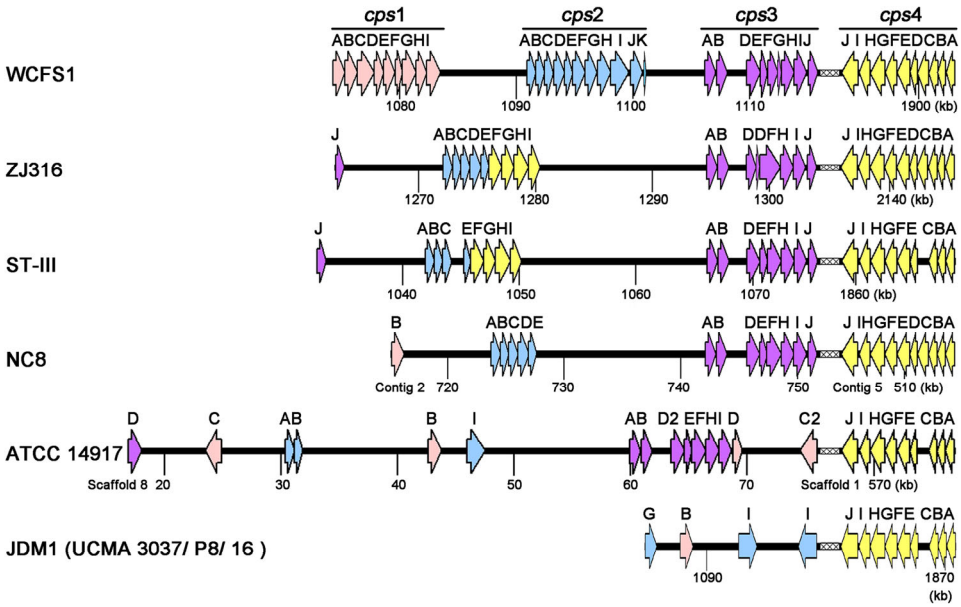

Fig. 4 Diversity of capsular polysaccharide biosynthesis genes in the completely sequenced genomes of *L. plantarum*

The four *cps* gene clusters are represented by pink, blue, purple, and yellow arrows, respectively (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

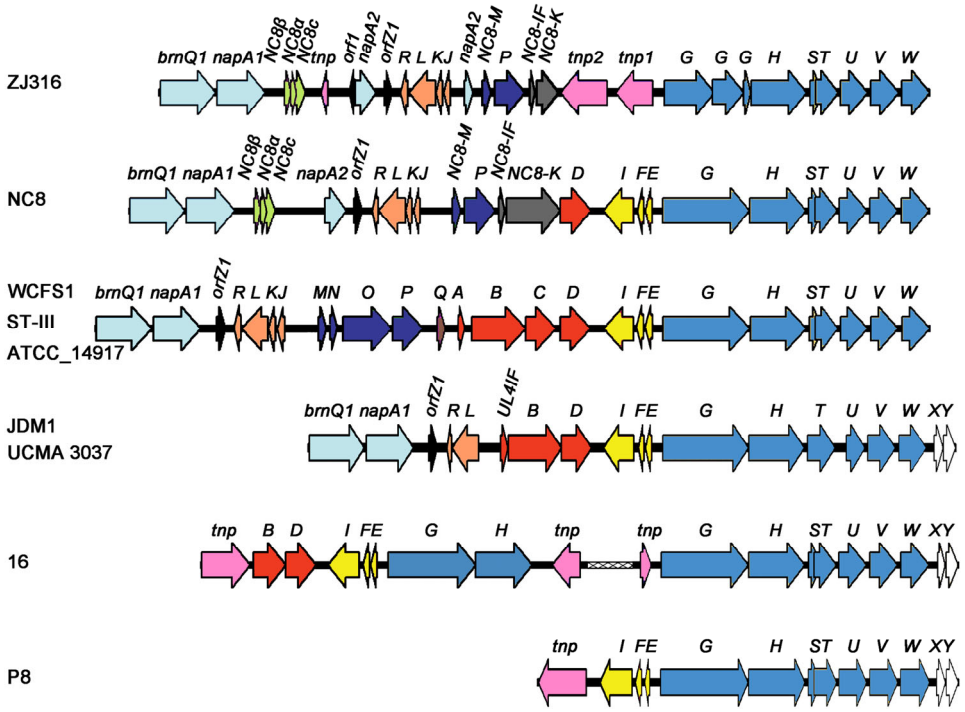

Fig. 5 Genetic map of the *pln* loci of different *L. plantarum* strains

The *pln* genes are represented by arrows with different colors corresponding to each operon. The arrows indicate that the genes are partial sequences according to those published in the GenBank database. Color codes for *pln* operons: red/grey for the genes of the regulatory operon; yellow for the *pln*EFI operon; blue for the *pln*MNOP operon; orange for the *pln*JKLR operon; pale green for the *pl*NC8βαc operon; gray-blue for the ABC transporter system genes; and purple for genes of the transposases. Strain-specific genes are named as open reading frames (ORFs) and are colored in white and black (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

However, there are no *pln*C or *pln*D response regulator orthologs or two-peptide bacteriocin *pln*EFI operons in ZJ316. The ZJ316 strain could produce at least two class IIb bacteriocins, PlnJK and PlnNC8βα, and a class IIc bacteriocin, PlnA, suggesting that this *L. plantarum* strain is a great producer of bactericins, which could explain its antimicrobial activity against pathogens.

3.9 CRISPR repeats

CRISPRs represent a family of DNA repeats that are typically composed of short and highly conserved repeats, interspaced by variable sequences called spacers and are often found adjacent to *cas* (CRISPR-associated) genes (Sorek *et al.*, 2008). CRISPRs represent the most widely distributed prokaryotic family of repeats and act as defense systems against invasion of foreign genetic material, in particular, phages (Barrangou *et al.*, 2007). *L. plantarum* ZJ316 contains one CRISPR locus, which belongs to the well-conserved Lsal1 family (Horvath *et al.*, 2009). This locus contains 7 perfect repeats of a 36-bp sequence (5'-GTCTTGAATAGTAGTCATATCAAAC AGGTTTAGAAC-3'). Four *cas* genes (zj316_0669–zj316_0672) are found upstream of the DNA repeats with an average G+C content of 42.2% (vs. 44.6% for the chromosome), suggesting that either these genes were acquired from an organism with a similar G+C content or were acquired in the distant past. The presence of CRISPR loci may increase the genome stability of strain ZJ316, and therefore its adaptation in the environment.

4 Conclusions

ZJ316 is a *L. plantarum* strain isolated from infant feces and has several great probiotic properties. In the present study, we revealed the genes that may be related to its genetic adaptation and probiotic profiles based on comparative genomic analysis. Genes related to carbohydrate transport and metabolism, proteolytic enzyme systems and amino acid biosynthesis, prophages and CRISPR adaptive immunity, stress responses, bile salt resistance, adhesion ability, CPS biosynthesis, and bacteriocin biosynthesis were identified. The feature of bacteriocin biosynthesis may provide clues to explain the antimicrobial activity of ZJ316. Bile salt resistance, adhesion ability, and stress resistances show its potential capability to survive in vitro environmental stresses and in vivo human GIT conditions. This research could provide the genetic basis for further studies elucidating the functional mechanisms of the probiotic properties of ZJ316 and facilitate its consideration as a potential probiotic candidate. Further research, such as transcriptomic and proteomic studies, will allow us to elucidate the physiological importance of the genes identified in bacterial survival, host colonization, pathogen exclusion, and antimicrobial activity.

Compliance with ethics guidelines

Ping LI, Xuan LI, Qing GU, Xiu-yu LOU, Xiao-mei ZHANG, Da-feng SONG, and Chen ZHANG declare that they have no conclict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 PTS predicted in *L. plantarum* ZJ316
Table S2 Peptidases and proteases genes in *L. plantarum* ZJ316

中文概要

题　目：比较基因组学揭示植物乳杆菌 ZJ316 的生境适应性及潜在益生特性

目　的：前期研究发现植物乳杆菌 ZJ316 能显著抑制病原菌，促进仔猪生长，提高猪肉质量等，本研究拟在 ZJ316 全基因组测序的基础上，运用比较基因组学手段揭示与其生境适应性及益生特性相关基因。

创新点：首次从基因水平上分析与植物乳杆菌 ZJ316 的生境适应性、抑菌活性及益生特性等相关的基因，为进一步揭示其生理功能打下基础。

方　法：运用 BLASTN、Mauve 和 MUMmer 等将植物乳杆菌 ZJ316 全基因组序列与已测序的 8 个植物乳杆菌全基因组序列进行比对及分析；用 CRISPRFinder 寻找 CRISPR 重复序列。

结　论：植物乳杆菌 ZJ316 包含碳水化合物的运输和代谢、蛋白水解酶系统和氨基酸的生物合成等相关基因，具有 CRISPR、应激反应、耐胆盐、粘附宿主肠壁、胞外多糖、生物合成和细菌素生物合成等相关基因。这些基因的功能是其作为益生菌的重要特征和基础。

关键词：植物乳杆菌 ZJ316；比较基因组学；益生菌；适应性