

Genomic insights and probiotic characteristics of *Bifidobacterium gallinarum* CACC 514 isolated from canine

Min Young Jung

Center for Industrialization of Agricultural and Livestock Microorganisms

Yangseon Kim (✉ yangseon@cialm.or.kr)

Center for Industrialization of Agricultural and Livestock Microorganisms

Jung-Ae Kim

Center for Industrialization of Agricultural and Livestock Microorganisms

Dae-Hyuk Kim

Chonbuk National University

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Abstract

Background: The genus *Bifidobacterium* includes common healthy gut microbes in mammals and is widely acknowledged as a probiotics with health-promoting properties.

Results: *Bifidobacterium gallinarum* CACC 514 was isolated from canine feces and its potential probiotic properties were characterized by comparative and functional genome analyses. The complete genome of strain CACC 514 was found to be 2.4 Mb, with a G + C content of 63.9 mol%. The strain possessed factors beneficial to mammalian health based on the presence of genes related to mucosal surface adhesion proteins, stress-related genes, and extracellular polysaccharide genes. A comparative genome analysis with other *Bifidobacterium* species revealed the unique characteristics of this species. These functional genomics have been confirmed for its superiority by probiotic properties, acid and bile tolerance, and adhesion to mucus.

Conclusions: The genome analysis and in vitro probiotics characteristics revealed its superiority in the intestine. These results add to our comprehensive understanding of *B. gallinarum* and suggest that this strain has potential application in mammalian probiotics. **Keywords:** *Bifidobacterium gallinarum*, gut microbe, probiotics, genome analysis, canine, feces.

Background

Bifidobacteria were first isolated from the feces of a breastfed infant and were characterized as gram-positive, non-motile, non-sporulating, anaerobic bacteria [1]. Bifidobacteria have been mostly isolated from the gastrointestinal tract of various mammals and birds, and more recently from food [2, 3]. The genus *Bifidobacterium* belongs to the lactic acid bacteria (LAB) group, which currently comprises over 70 species described in the List of Prokaryotic Names with Standing in Nomenclature (LPSN, 2019).

Bifidobacteria form a significant proportion of the gut microbiota in animals and humans. Bifidobacteria are used as probiotics, and exert various health beneficial properties, such as antimicrobial activity against pathogenic bacteria, reduction of cancer, and prevention or treatment of inflammatory bowel disease (IBD) and diseases related to IBD including Crohn's disease (CD) and ulcerative colitis (UC) [4–7].

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' retaining the previous definitions by Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO, 2002)[8]. To benefit health, probiotic organisms require to have therapeutic effects, including gastric acid and bile compounds stability, adherence to the intestinal surface, and colonization to the intestinal tract [9]. Probiotics lactic acid bacteria were associated with intestinal barrier function by their metabolic products, such as short-chain fatty acids [10]. In particular, there has been a continuing report on the strong association between *Bifidobacterium* numbers and enhanced integrity and invasiveness of the intestinal epithelial cell barrier [11], and thus, enteric adhesion studies for this strain are important.

The genomes of several probiotic *Bifidobacterium* strains including *B. longum* subsp. *infantis*, *B. bifidum*, and *B. animalis* subsp. *lactis* have been sequenced and analyzed in efforts to identify the genes and metabolic pathways related to their health-promoting traits [12–14]. However, considering the importance of biotechnology, the number of strains sequenced completely so far is still small and is largely limited to phylogenetic studies due to sampling bias for the *Bifidobacterium* species mentioned above [15]. The recognized taxa within the genus *Bifidobacterium* are divided into six main phylogenetic groups namely, *B. asteroides*, *B. adolescentis*, *B. longum*, *B. pullorum*, *B. pseudolongum*, and *B. boum* [16]. The genome of *B. pullorum* group species is also not completely analyzed.

In the present study, we isolated the *B. gallinarum* species (strain CACC 514) within the *B. pullorum* group from canine feces, and to the best of our knowledge, we identified its first complete genome sequences. The basic requirements of probiotic applications include resistance to gastrointestinal (GI) stress, the ability to colonize the gastrointestinal tract and to be metabolically active in the gastrointestinal tract [17]. The complete sequences of the CACC 514 genome were annotated and compared with the genomes of other *Bifidobacterium* strains to determine the basis of its potential and unique probiotic traits. Furthermore, *in vitro* experiments for survival against gastric intestinal tract confirmed the positive effects as probiotics.

Results And Discussion

General genome features of Bifidobacterium gallinarum CACC 514

The complete genome of *B. gallinarum* CACC 514 contained a single circular chromosome of 2,414,462 bp with a GC content of 63.9% and one plasmid (8,720 bp) with an average GC content of 64.2 % (Table 1 and Fig. 1A). In total, 1,957 protein-coding sequences (CDSs) were identified. The chromosome contained 9 rRNAs, 57 tRNAs, 3 other ncRNAs, and 58 pseudogenes. The G+C contents belong to genus *Bifidobacterium* (55–67 %) were similar to the values 64.2 % of *B. gallinarum* DSM 20670^T with 2.1 Mb genome size, 5 rRNAs and 53 tRNAs [15, 18, 19].

Functional classification

Each CDS in the annotated genome was grouped into the RAST subsystem category based on the predicted functional role (Additional File 1). Among the 2,102 CDSs, only 774 CDSs could be categorized into RAST subsystems, representing 36.8% of the total CDSs. Among the 2,102 categorized CDSs, the majority were classified into subsystems of protein metabolism (172 CDSs, 22.2%), amino acids and derivatives (128 CDSs, 16.5%), carbohydrates (117 CDSs, 15.1%), and DNA metabolism (64 CDSs, 82.7%). The remaining portion (63.2%) could not be classified into any subsystem categories. Furthermore, 1,720 CDSs were specifically classified to a cluster of 19 COG-based functional categories (Fig. 1B). Most genes were classified into functional categories for carbohydrate transport and metabolism (199 genes), replication, recombination, and repair (163 genes), translation, ribosomal structure, and biogenesis (135 genes), amino acid transport and metabolism (128 genes), and transcription (112 genes).

PHAST

The PHAGER search tool identified 1 intact prophage and 2 incomplete phages in this genome (Additional file 2). One prophage region resembled Microb_Min1_NC_009603 (42.7 kb, region 1) with a GC content of 62.3%. Bacteriophages are the most abundant biological entities and are known to impact genomic evolution and the adaptive capabilities of their bacterial hosts [20]. Bifidoprophages are present in a small number in multiple bifidobacterial species, indicating their relatedness to phages infecting other *Actinobacteria*, *Firmicutes*, as well as gram-negative bacteria [21, 22]. In addition, integrases are useful indicators of prophage diversity in bacterial genomes [23]. Prophage region 1 (804,476 - 847,197 bp) contained 58 CDSs, with a complete prophage integrase gene (Additional file 3). Consistent with the findings reported for bifidoprophages [22], the most conserved modules of the *B. gallinarum* CACC 514 were DNA packaging (encoding terminase, portal, and capsid proteins), lysogeny (encoding integrase gene), and tail morphogenesis module (encoding the tape measure protein).

CRISPR

The *B. gallinarum* CACC 514 genome contained 9 CRISPR loci (CRISPR 1 to 9), including 2 confirmed CRISPRs (3 and 4), and 7 questionable CRISPRs (1, 2, and 5–9) (Additional file 4). The strain CACC 514 was identified as a Type II-A system. *Bifidobacterium* species have unusually large and diverse CRISPR-Cas systems that constitute an adaptive immune system for antiviral defense in bacteria. CRISPR-Cas Type II immune systems (II-A, II-C) are fairly rare and occur in only 5% of bacteria, but they occur at a much higher frequency in the *Bifidobacterium* genus [24]. Characterization of Type II elements may provide opportunities to use the molecular genome-editing tool for the development of next-generation probiotic bacteria [25]. The *B. gallinarum* CACC 514 CRISPR-Cas system may also provide a platform for various potential biotechnological and ecological uses as probiotic bacteria.

Stress-related proteins

A number of stress-related proteins can regulate the adaptability of bacteria to the gastrointestinal tract [26]. CACC 514 possesses genes involved in stress-related pathways (Table 2). These stresses include temperature, pH, and oxidative stress. CACC 514 carried various genes encoding heat shock proteins, *groEL*, *groES*, *dnaJ*, *dnaK*, *grpE*, *clpB*, *clpC*, and *clpP*, and the small heat shock proteins (sHsps), *hsp20* (Table 2). These genes are induced to respond upon exposure of bifidobacterial cultures to stressful condition, and overexpression of sHsps is known to increase tolerance to heat and osmotic stress in *B. breve* and *B. longum* NCC2705 [27]. CACC 514 encodes 2 genes for sodium-proton antiporter (Na⁺/H⁺), providing evidence of tolerance to low pH in the GI environment [23]. CACC 514 contains 9 genes for antioxidative proteins that are involved in minimizing the toxicity of active oxygen species (Table 2). *B. longum* has NADH oxidase, NADH peroxidase, and low superoxidase dismutase, whereas CACC 514 contains an NADH peroxidase and only 1 other predicted protein anaerobillin synthase (*chuW*) that is not found in *Bifidobacterium* species [28].

Extracellular proteins

Potentially surface exposed (PSE) proteins and cell envelope-associated structures play crucial roles in establishing and maintaining interactions between the microbe and host epithelium [16]. All previously sequenced *Bifidobacterium* strains appear to encode an extracellular polysaccharide (EPS) or capsular polysaccharide. CACC 514 also encodes EPS (*epsF*, *epsL*, *epsH*, and *epsJ*), and capsular polysaccharide (ABC-2.CPSE.A) (Table 2). These may contribute useful functions in the host such as adhesion, nutrient availability, pathogen inhibition, and immune system modulation in the bifidobacteria [29, 30].

Phylogenetic analysis

CACC 514 showed 99.2% 16S rRNA gene sequence similarity with *B. gallinarum* DSM 20670^T and *B. pullorum* DSM 20433^T, and less than 97.0% similarity with *B. saeculare* DSM 6531^T and *B. longum* subsp. *infantis* ATCC 15697^T, respectively (Additional file 5). The 96.5% ANI (96.5%) and 71.4% *in silico* DDH values revealed that the strain CACC 514 belongs to the same species as *B. gallinarum* DSM 20670^T with higher values than the ANI and DDH cut-off values (Fig. 2) [31].

Core- and pan-genomes of CACC 514 and Bifidobacterium pullorum group strains

The complete CACC 514 genome was compared with the complete and incomplete genomes of 6 *Bifidobacterium* strains (Additional file 6). The genome of *B. pullorum* group strains could not be compared in this study, as there are no complete genome data reported in NCBI. These 7 genomes composed a pan-genome of 4,526 orthologous gene families and a core genome of 1,088 orthologous gene families (Additional file 7). In total, 271 genes in CACC 514 were unique (Additional file 8), including 236 hypothetical proteins. The 35 specific genes of CACC 514 included CRISPR-Cas 2, carbohydrate metabolism, phages, and cell wall and capsular polysaccharide-related genes. These outcome proteins will provide a higher ability for environmental change adaptation and adhesion to intestinal epithelial cells.

Probiotics Properties

Acid and Bile tolerances and intestinal adhesion

The acid and bile tolerance help in studying the survival of strain under low pH gastric juice condition and colonization of isolates in the small intestine [32]. The strain showed a high survival rate (%) above 80% at low pH, and 0.3 and 1% bile salt concentration after 2h exposure (Table 3). The CACC 514 with 82.80% adhesive ability to HT-29 cells was higher than the reference strain *L. rhamnosus* GG (Table 4).

Antibiotic susceptibility

The susceptibilities to the tested 12 antibiotics of CACC 514 was very susceptible to amoxicillin/clavulanic acid, ampicillin, clindamycin, imipenem, metronidazole, tetracycline, vancomycin and erythromycin, which showed MICs ≤ 4 $\mu\text{g/ml}$ (Table 5). These results were similar with the other probiotics *Bifidobacterium* species, *B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve*, and *B. longum* [33]. *Bifidobacterium* spp. had a high resistant to kanamycin above 500 $\mu\text{g/ml}$ concentration [34], and the

most have been reported as resistant to aminoglycoside antibiotic, because of the absence of cytochrome mediated drug transport system [35]. CACC 514 showed also the resistance to kanamycin ($\geq 256 \mu\text{g/ml}$) and aminoglycoside gentamycin ($96 \mu\text{g/ml}$). These antibiotic susceptibilities were evaluated comparing MIC values to breakpoints suggested by European Food Safety Authority (EFSA) [36].

Phenotypic (Fermentative) profiling

The CACC 514 was utilized D-Glucose , mannose, mannitol, lactose, sucrose, maltose, salicin, xylose, arabinose, and raffinose was utilized. Esculin is hydrolyzed and nitrate is not reduced. The strain showed positive reactions for α -arabinosidase, esterase, leucine arylamidase, valine arylamidase, cystine arylamidase, alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, α -glucosidase, and β -glucosidase.

Conclusions

Bifidobacterium is important in gut microbiome studies and has long been used as a probiotic to provide consistent beneficial health effects. Genomic analysis of *B. gallinarum* CACC 514 isolated from canine feces provided an overview of the potential mechanisms underlying the effect of the strain on host health. The specific genes of CACC 514 including CRISPR-Cas 2, carbohydrate metabolism, phages, and cell wall and capsular polysaccharide-related genes will provide higher adhesion to intestinal epithelial cells. In addition, *in-vitro* probiotic properties, low pH and bile resistance, and high adhesion to intestinal epithelial cells of CACC 514 validated genomic properties. Taken together, these studies demonstrate the potential as a probiotic of *B. gallinarum* strains within the *B. pullorum* group and substantially support the safe use of CACC 514 as a probiotic in the animal industry.

Methods

Isolation of bacteria

CACC 514 was isolated in Korea from dog feces (brown poodle, 4.8 years of age, male). Serial 10-fold dilutions of the fecal sample were prepared and cultivated on modified MRS (mMRS, de Man Rogosa and Sharpe with 0.05% cysteine-HCl). Plated samples were incubated in an anaerobic atmosphere (5% hydrogen and 5% carbon dioxide, and 90% nitrogen) for 48 h at 37 °C.

DNA extraction and sequencing

Genomic DNA was extracted using a DNeasy UltraClean microbial kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The isolated DNA was sequenced using single molecular real-time (SMRT) sequencing technology with C4 chemistry and P6 DNA polymerase on the PacBio RS II system (Pacific Biosciences, Menlo Park, CA, United States) and Illumina HiSeq system (Illumina, San Diego, CA, United States) at MacroGen Inc. (Seoul, Republic of Korea).

Annotation

De novo assembly of the single molecule real-time sequencing reads was performed using the hierarchical genome assembly process workflow (HGAP 3.0) in PacBio's SMRT portal with subreads from PacBio. After assembly, the paired-end reads from Illumina HiSeq 2500 were mapped to the assembled contigs to improve the accuracy of the genome sequences [37]. The sequences were annotated using the combined results of the automated NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) and the RAST prokaryotic genome annotation server (<http://rast.nmpdr.org/>) [38]. The coding genes were predicted according to clusters of orthologous group (COG) using the WebMGA on-line tool [39]. Prophage insert regions were searched using the online phage search tool PHASTER (<http://phaster.ca/>), and clustered regularly interspaced short palindromic repeats (CRISPR) were predicted using the CRISPR web server (<http://crispr.i2bc.paris-saclay.fr/>) [40, 41].

Phylogenetic analyses of *Bifidobacterium* strains

Phylogenetic analysis was performed based on the 16S rRNA gene sequences. The 16S rRNA gene sequences of the CACC 514 and related species were aligned using the multiple sequence alignment program CLUSTAL W, and phylogenetic trees were then constructed using the neighbor-joining algorithm based on 1000 bootstrap replications in MEGA version 7 [42]. The ANI and in silico DDH values between *Bifidobacterium* strains were calculated using a standalone software (<http://www.ezbiocloud.net/sw/oat>) [43] and genome-to-genome distance calculator 2.1 (GGDC, <http://ggdc.dsmz.de/distcalc2.php>) [44], respectively.

Pan-genome comparison

Seven whole genome sequences from the *B. pullorum* group within the same clade in the phylogenetic tree of 16S rRNA gene and sequences of *B. longum* were selected for whole genome phylogenetic and comparative analysis. *Bifidobacterium gallinarum* DSM 20670 (GCA_000771505.1), *B. pullorum* DSM 20433 (GCA_000771405.1), *B. saeculare* DSM 6531 (GCA_000770965.1), and *B. eulemuris* DSM 100216 (GCA_002259685.1). The two *B. longum* subsp. *longum* JCM 1217 (GCA_000196555.1) and *B. longum* subsp. *infantis* ATCC 15697 (GCA_000020425.1) were used as the out-group in the phylogenetic analysis. The present sequenced genome, *B. gallinarum* CACC 514 was used for comparative analysis.

Pan-genome Orthologous Groups (POGs) were analyzed using BIOiPLUG comparative genomics software (ChunLab Inc., Seoul, Republic of Korea), and a heat map, UPGMA dendrogram, and Venn diagram were constructed based on these data.

Quality assurance

A single colony of the *B. gallinarum* strain CACC 514 was repeatedly transferred to fresh mMRS medium to obtain pure cultures and the identity of the strain was verified through 16S rRNA gene sequencing. Genomic DNA strain CACC 514 was extracted using Qiagen DNeasy UltraClean microbial kit and then confirmed through a BLAST search of the 16S rRNA gene.

Probiotic characteristics

Acid and bile tolerance

Acidic pH resistance were assessed using MRS broth adjusted to pH 1.5 and 2.5 with 6N HCl and incubated at 37 °C for 30min, 1h and 2h. Bile tolerance was evaluated with MRS broth containing 0.3% (w/v) oxgall (BD Difco, USA) at 37 °C for 2h. Viable number of bacteria were enumerated using MRS agar plate.

Adhesion assay

The human colon adenocarcinoma HT–29 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco BRL, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100mg/ml). Cells were incubated with DMEM medium lacking antibiotics before adhesion assay. LAB cultures suspended in DMEM medium without FBS and antibiotics were added into the wells containing HT–29 cell. The plates were incubated at 37 °C for 2h under 5% CO₂ and then the adherent bacteria were counted by plating the serial 10-fold dilution of the suspensions using MRS agar plate. The *L. rhamnosus* GG (LGG, KCTC 5033) was used as reference strain. All probiotic activity were repeated three and results expressed as mean ± standard deviation.

Antibiotic susceptibility

The MICs (µg/mL) of eleven antibiotics were determined using commercial E-test® (Epsilometer test, bioMerieux, France): amoxicillin/clavulanic acid, ampicillin, clindamycin, gentamicin, impenem, kanamycin, metronodazole, tetracycline, vancomycin, erythromycin, doxycycline and trimethoprim/sulfadiazin. The concentration on the strips was from 0.016 to 256 µg/mL with the exception of impenem and trimethoprim/sulfadiazin (0.002–32 µg/mL). Antibiotic susceptibility assay was performed according to methods described [45]. The MIC results was interpreted according to the criteria documented in the E-test technical guide.

Phenotypic (Fermentative) profiling

The sugar degradation and other enzyme properties of the CACC 514 strain were characterized by using an API 20A, API Rapid ID 32A, and API ZYM kit (Bio-Merieux, Marcy l’Etoile, France). The strain was grown until the logarithmic phase and then inoculated into API galleries according to the manufacturer’s instructions.

List Of Abbreviations

CRISPR, clustered regularly interspaced short palindromic repeats; COG, clusters of orthologous group; CD, Crohn’s disease; EPS, extracellular polysaccharide; GI, gastrointestinal; IBD, inflammatory bowel disease; LAB, lactic acid bacteria; POGs, Pan-genome Orthologous Groups; PSE, potentially surface

exposed; PGAP, Prokaryotic Genomes Annotation Pipeline; CDSs, protein-coding sequences; SMRT, single molecular real-time; UC, ulcerative colitis

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The complete genome of strain CACC 514 determined in this study has been deposited with the NCBI GenBank database under accession numbers CP035464 (chromosome) and CP035465 (plasmid).

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

MYJ and YK wrote the manuscript. MYJ, JAK, and DHK performed DNA preparation, gene annotation, and comparative genome analysis. All authors read and approved the final manuscript.

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Tables

Table 1. Overview of the *Bifidobacterium gallinarum* CACC 514 genome

Attribute	Chromosome	Plasmid
Size (Mb)	2.41	0.01
GC%	63.9	64.2
Protein	1,951	6
rRNA	9	-
tRNA	57	-
Other RNA	3	-
Pseudogene	58	-
Accession No.	CP035464	CP035464

Table 2. Genes related to stress adaptation and adhesion in *B. gallinarum* CACC 514

	CDS name	Other name(s)	Product	Length	Location
Temperature	gene_00157	HtpX	Protease HtpX like protein	957	204082..205038
	gene_00307	ClpB	Chaperone protein ClpB	2652	397922..400573
	gene_00759	ClgR	Transcriptional regulator ClgR	513	959703..960215
	gene_00799	clpP CLPP	Endopeptidase Clp	612	1016297..1016908
	gene_00800	clpP CLPP	Endopeptidase Clp	702	1016928..1017629
	gene_00832		Acid shock protein; small heat shock protein (HSP20) family	456	1050933..1051388
	gene_01069	HrcA	Heat-inducible transcription repressor HrcA; grpE- dnaK-dnaJ and groELS operons	1098	1301525..1302622
	gene_01070		Chaperone protein DnaJ	1146	1302676..1303821
	gene_01501		ATP-dependent Clp protease ATP-binding subunit ClpA like protein CD4A, chloroplastic	2541	1713433..1715973
	gene_01834		10 kDa chaperonin; GroES chaperonin family	294	2095638..2095931
	gene_02064	HspR	Putative heat shock protein HspR	573	2386201..2386773
	gene_02065	DnaJ	Chaperone protein DnaJ	1008	2386940..2387947
	gene_02066		Protein GrpE	723	2388049..2388771
	gene_02067		Chaperone protein dnaK1	1866	2388787..2390652
pH	gene_00100		Sodium, potassium, lithium and rubidium/H(+) antiporter	2076	130424..132499
	gene_00805	NhaA	Na(+)/H(+) antiporter NhaA	1314	1020818..1022131
Oxidative stress	gene_00032	trxB TRR	Thioredoxin-disulfide reductase	1770	46079..47848
	gene_00033	PRDX2_4 ahpC	Peroxiredoxin	564	47979..48542
	gene_00064	trxB TRR	Thioredoxin-disulfide reductase	933	81630..82562
	gene_00211	BCP PRXQ DOT5	Peroxiredoxin	489	274591..275079
	gene_00346		uncharacterized protein	957	446729..447685
	gene_00423		Protein disulfide-isomerase	360	557893..558252
	gene_01016	chuW	Anaerobillin synthase	1260	1232597..1233856
	gene_01422	npr	NADH peroxidase	1746	1613981..1615726
	gene_01489	trxB TRR	Thioredoxin-disulfide reductase	255	1698312..1698566
	gene_01664	fadH	2,4-dienoyl-CoA reductase (NADPH)	2010	1913824..1915833
Extracellular polysaccharide (EPS)	gene_00388	epsF	Putative glycosyltransferase EpsF	1098	506424..507521
	gene_00389	epsF	Putative glycosyltransferase EpsF	1110	507505..508614
	gene_00413	epsL	Uncharacterized sugar transferase EpsL	1626	541499..543124
	gene_00385	waaH EpsH	Putative glycosyltransferase EpsH	1059	502775..503833
	gene_00390	amsB EpsJ	Uncharacterized glycosyltransferase EpsJ	1002	509807..510808
	gene_01984	waaV EpsH	Putative glycosyltransferase EpsH	1038	2277617..2278654
Capsular polysaccharide	gene_00825	ABC-2.CPSE.A	Capsular-polysaccharide-transporting ATPase	744	1044643..1045386

Table 3. Acid and bile tolerance of *B. gallinarum* CACC 514

	Viable cell count (Log CFU/ml)		
	Acid tolerance	Bile tolerance	
	pH2.5	0.3% oxgall	1% oxgall
0h	7.97 ± 0.02	7.97 ± 0.02	7.97 ± 0.02
2h	6.84 ± 0.16	7.17 ± 0.01	6.63 ± 0.09
Survival rate(%)	85.77	89.92	83.13

Table 4. Intestinal adhesion activity of *B. gallinarum* CACC 514

	Viable cell count (Log CFU/ml)	
	CACC 514	<i>L. rhamnosus</i> GG
0h	7.88 ± 0.13	7.63 ± 0.23
2h	6.52 ± 0.07	6.25 ± 0.11
Adherence (%)	82.8	81.93

Table 5. Antibiotic susceptibility of *B. gallinarum* CACC 514. Bacterial susceptibility to antibiotic was determined according to the cut-off values given by EFSA (2012). R, resistant; n.r, not recommended.

Antibiotics	Minimal inhibition concentraion (MIC, µg/mL)	
	CACC 514	EFSA cut-off
Amoxicillin/Clavulanic acid (XL)	0.125	n.r
Ampicillin (AM)	0.094	2
Clindamycin (CM)	0.016	1
Gentamicin (GM)	96	64
Impenem (IP)	0.5	n.r
Kanamycin (KM)	>256 ^R	n.r
Metronidazole (MZH)	4	n.r
Tetracycline (TC)	4	8
Vancomycin (VA)	1	2
Erythromycin (EM)	0.032	1
Trim/Sulfa (TS)	0.032	n.r
Doxycycline(DC)	4	n.r

Figures

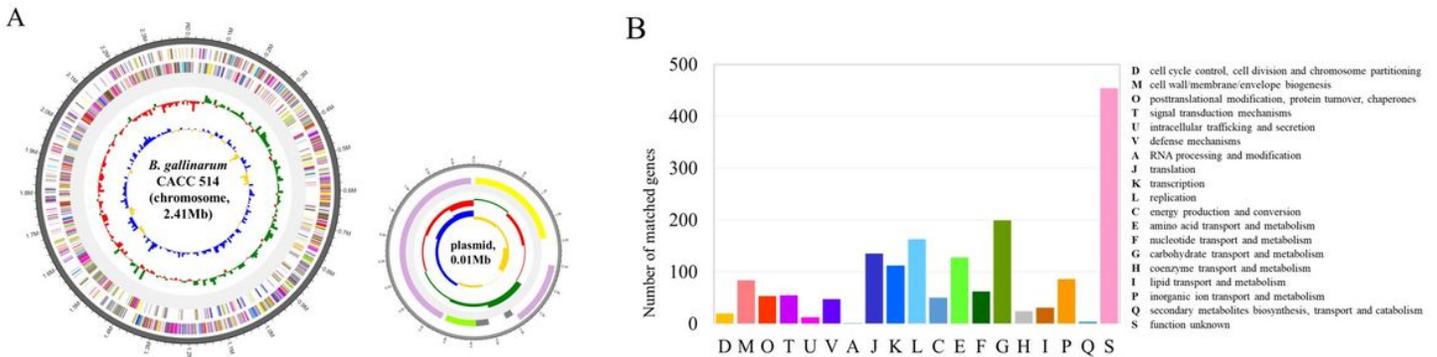


Figure 1

Genome features of *Bifidobacterium gallinarum* CACC 514. (A) Circular genome maps of *Bifidobacterium gallinarum* CACC 514 chromosome and plasmid. Circles from the outside to the center denote rRNA and tRNA genes, reverse strand CDS, forward strand CDS, GC skew, and GC content. (B) Genome number of COG categories.

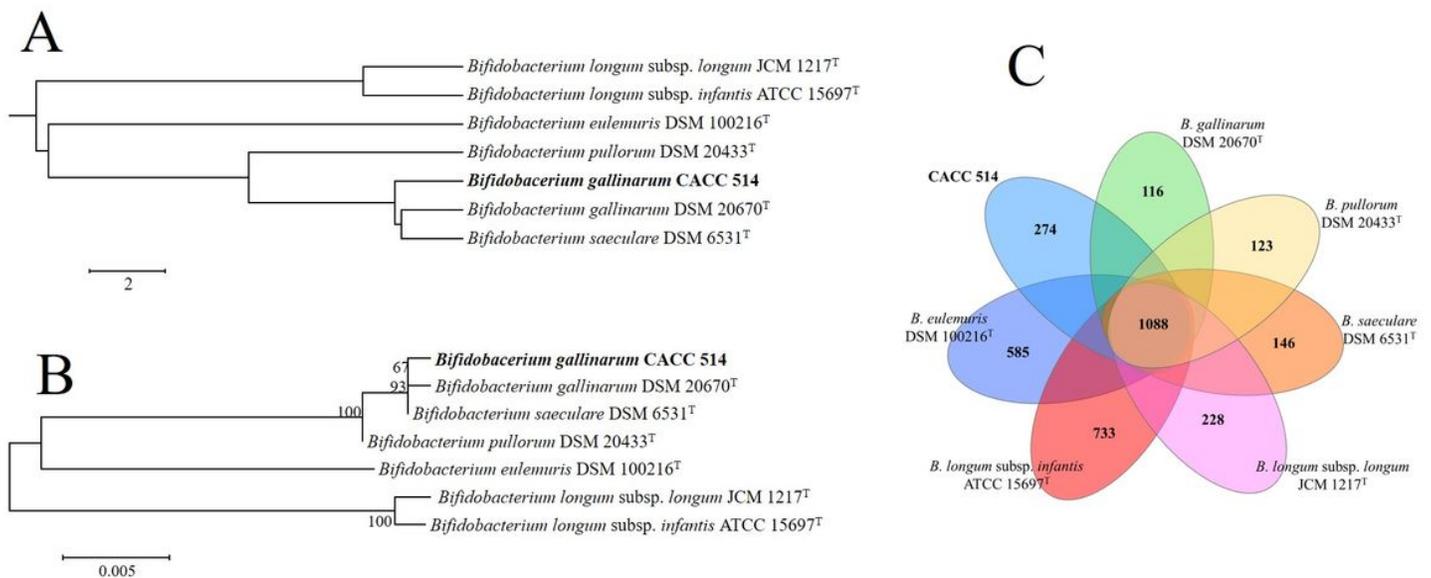


Figure 2

Phylogenetic tree based on average nucleotide identity (ANI, A) and in silico DNA-DNA hybridization (DDH, B) values showing pair-wise relatedness between 7 *Bifidobacterium* strains. (C) Venn diagram showing

the number of orthologous gene groups of the core genome (the center part) and the unique genes of each genome among 7 Bifidobacterium strains.

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