Current Applied Science and Technology Vol. 22 No. 5 (September-October 2022)

Research article

Characterization, Genome Analysis and Probiotic Properties of L-Lactic Acid Producing *Enterococcus lactis* FM11-1

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Received: 21 July 2021, Revised: 7 December 2021, Accepted: 18 January 2022

DOI: 10.55003/cast.2022.05.22.012

Abstract

Keywords

Enterococcus lactis;
flower;
genome analysis;
polyphasic taxonomy;
probiotics

Strain FM11-1 was isolated from flowers of *Solanum torvum* that were collected in Nakhon Si Thammarat Province, Thailand and was characterized using a polyphasic approach as Gram-positive, facultatively anaerobic with short chains and catalase-negative cocci. This strain produced L-lactic acid from glucose and was closely related to *Enterococcus durans* NBRC 100479^T, *Enterococcus faecium* NRIC 1145^T, *Enterococcus hirae* ATCC 9790^T, *Enterococcus lactis* LMG 25958^T and *Enterococcus ratti* DSM 15687^T (98.92-99.73 %) based on 16S rRNA gene sequence similarity. The draft genome of strain FM11-1 had 2,784,928 bp and contained 2,586 coding sequences, with a genomic G+C content of 38.07 mol%. Values of ANIb, ANIm and digital DNA-DNA hybridization (dDDH) between strain FM11-1 and the closest strain, *E. lactis* LMG 25958^T were 97.23%, 98.30% and 83.7%, respectively. The predominant cellular fatty acids were $C_{19:0}$ cyclo ω8c and $C_{16:0}$. This strain was identified as *Enterococcus lactis* using polyphasic characterization and genome analysis. Strain FM11-1 contained genes involved in carbohydrate fermentation and specialty genes of antibiotic resistance. This strain showed adhesion ability (0.43±0.11) on Caco-2 cells but had no cytotoxicity against Caco-2, HepG2 and Vero cells.

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1. Introduction

Enterococci, a group of lactic acid bacteria (LAB), are Gram-positive and facultatively anaerobic with catalase-negative cocci [1]. The genus *Enterococcus* consists of 59 species with validly published names (https://lpsn.dsmz.de/genus/enterococcus). *Enterococcus* strains are commonly found in a wide variety of environments, including the gastrointestinal tract of humans and animals, feces, soil, water, plants and fermented food originating from meat, dairy and vegetable sources. *Lactobacillus* and *Pediococcus* strains are commonly used in food fermentation and play a beneficial role in dairy products and fermented foods as probiotics administered to reduce the risk of irritable bowel syndrome (IBS), prevent diarrhea, alleviate cholesterol levels and modulate immunity.

Members of the genus *Enterococcus,* particularly *E. faecium*, *E. faecalis* and *E. lactis* strains are utilized as probiotics and beneficially applied in the food, cosmetic and pharmaceutical industries and medicines [2-6]. The candidate probiotic *Enterococcus* strains ES11, ES20 and ES32 have also been reported to tolerate acid and bile salts, adhere to intestinal Caco-2 cell line, and provide cholesterol-lowering properties and anti-pathogenic activity [7]. *Enterococcus lactis* RS5 resisted simulated gastrointestinal conditions and showed anti-pathogenic activity [8]. *Enterococcus lactis* PMD74 endured protease enzymes and the simulated gastrointestinal environment, while its virulence determinant (*esp*) was negative [9]. *Enterococcus lactis* YHC20 exhibited cholesterol-lowering effects and probiotic attributes including acid and bile tolerance, bile salt hydrolase (BSH) activity and cell surface hydrophobicity [10]. *Enterococcus lactis* Q1 and 4CP3 resisted gastric and digestive enzymes and were sensitive to antibiotics but the virulence factors hemolysin and gelatinase were not detected [11].

For decades, bacterial identification has relied on polyphasic approaches including phenotypic, chemotaxonomic and genotypic characteristics. However, the use of the 16S rRNA gene sequence is limited and does not allow identification of *Enterococcus* at the species level because of the high sequence similarities between some species [12-14]. Recently, genome analysis has become useful technique for enhancing bacterial identification, and it offers various advantages over Sanger sequencing with high resolution and accuracy [15, 16]. In this study, the strain FM11 isolated from the flowers of *Solanum torvum* was found to exhibit tolerance in medium at pH 2 and 0.3% bile including bile salt hydrolase and cholesterol assimilation activity [17]. The study of polyphasic taxonomy, genome analysis and probiotic properties of this strain proved interesting. The draft genomic analysis enhanced the accuracy of identification and also provided specialty gene information between related species.

2. Materials and Methods

2.1 Isolation of strain

Flowers of *Solanum torvum* (Family *Solanaceae*) were collected from Nakhon Si Thammarat Province in Southern Thailand. Approximately 0.5 g of each sample was enriched in MRS broth (de Man, Rogosa and Sharpe; Difco) [18] and incubated at 37°C under aerobic conditions for 48-72 h. After incubation, one loopful of culture broth was streaked on MRS agar plates supplemented with 0.3% $CaCO_3$. A single colony surrounded with a clear zone was selected and purified on MRS agar plates at 37°C for 48 h. After purification, the pure culture was preserved in 10% skim milk at -80°C and lyophilized. The strain was deposited in culture collections with accession numbers JCM 33322, LMG 31283 and TISTR 2660.

2.2 Phenotypic and chemotaxonomic characterization

Cell shape, size, arrangement and colonial appearance of the strain were observed in cells grown on MRS agar plates for 2 days. Gram staining was performed as described by Barrow and Feltham [19]. Catalase activity, nitrate reduction, gas production, hydrolysis of aesculin, arginine, starch and slime formation were determined as previously reported [20]. Growth at different pH (3.5-10.0, using relevant buffers), temperatures (10, 15, 30, 37, 40, 42 and 45°C) and NaCl concentrations (1, 3, 5, 6, 6.5, 7, 7.5 and 8%, w/v) were evaluated in MRS broth. Diacetyl production was determined as reported by Phalip *et al*. [21]. Acid production from carbohydrates and enzyme activity was evaluated using API 50 CH and API ZYM (bioMérieux) according to the instructions of the manufacturer. Hemolytic activity was determined as previously described [19]. Lactic acid isomer was analyzed by the enzymatic method as described by Okada *et al*. [22] using high-performance liquid chromatography [23]. The fatty acid composition in freeze-dried cells obtained from 2-day-old cultures grown in MRS broth at 37°C was analyzed according to the instructions of the Microbial Identification System (MIDI) [24, 25].

2.3 Genotypic and genomic characterization

Genomic DNA was obtained from cells grown in MRS broth for 48 h [26, 27]. The 16S rRNA gene was amplified by PCR technique and sequenced (Macrogen, Seoul, Korea) using the following universal primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3'), 337F (5'-GACTCCTACGGGAGGCWGCAG-3'), 518F (5'-CCAGCAGCCGCGGTAATACG-3'), 800R (5'-TACCAGGGTATCTAATCC-3'), 1100R (5'-GGGTTGCGCTCGTTG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [28]. A phylogenetic tree was constructed based on the neighbor-joining method [29] in MEGA 7 software [30]. Confidence values of nodes were evaluated using the bootstrap resampling method with 1000 replications [31].

Draft genome sequences of strains FM11-1 and *E. lactis* LMG 25958^T were carried out using the Nextera DNA Flex Library Prep Kit and Illumina MiSeq platform with MiSeq v3 reagent kit (600 cycles). Genome annotation was performed using the DFAST web service [32]. Average nucleotide identity (ANI) values of strains FM11-1 and *E. lactis* LMG 25958^T, together with their closely related type strains were pairwise calculated using ANI-Blast (ANIb) and ANI-MUMmer (ANIm) algorithms [33] implemented within the JSpeciesWS web service [34]. Digital DNA-DNA hybridization (dDDH) was calculated using the Genome-to-Genome Distance Calculator (GGDC 2.1) by the BLAST+ method [35]. The phylogenomic tree based on whole-genome sequences was constructed on the Type (Strain) Genome Server (TYGS) [36]. The tree was inferred using FastME 2.1.4 [37] with Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. Branch lengths were scaled in terms of GBDP distance formula d_5. Results were based on recommended formula 2 (identities/HSP length), which is liberated of genome length and thus effective in the case of incomplete draft genome. Specialty genes prediction was carried out using the PATRIC web server [38].

2.4 Screening of probiotic property

2.4.1 Adhesion assay

An assay of adhesion ability to the intestinal epithelium was conducted as described by Fernández *et al*. [39] and Bustos *et al*. [40] with slight modifications. In brief, the Caco-2 cell concentration (5×10^5 cell/ml) was inoculated into 24-well tissue culture plates and incubated at 37°C in 5% CO_2 for 15 days. The culture medium was changed every 72 h. The overnight cells of strain FM11-1 in

MRS broth were collected by centrifugation at 4°C (14,000 rpm) for 10 min and washed twice with phosphate buffer solution (PBS). Then, the FM11-1 cells (10^8 CFU/ml) were re-suspended in DMEM containing 10% fetal bovine serum (FBS). The cell suspension was inoculated into Caco-2 cells in each well and incubated for 90 min at 37°C in 5% CO_2. Subsequently, the cells were washed three times with PBS and then lysed with Triton-X solution 0.05% (v/v). After incubation at 37°C for 10 min, the solution with released bacterial cells was serially diluted, spotted on MRS agar and incubated at 37°C for 48 h. Adhesion ability was reported as the percentage ratio between the number of adherent cells to Caco-2 cells and the total number of bacterial cells inoculated (CFU/ml). *Lactobacillus rhamnosus* GG was used as a positive control.

2.4.2 Sample preparation for cytotoxicity assay

Cytotoxicity against Caco-2 (colon carcinoma, ATCC HTB-37), HepG2 (human hepatocyte carcinoma, ATCC HB-8065) and Vero (normal cells from African monkey kidney) cells were conducted by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) based on the activity of active mitochondrial enzymes [41, 42]. In brief, cells were cultivated in MRS broth at 37°C for 48 h and then diluted in MRS broth (10^8 cells/ml). The cell-free supernatant (CFS) of the strain was collected by centrifugation at 7,000 rpm for 10 min and the pH was adjusted to neutral with 1 N NaOH before cytotoxicity testing. The CFS was concentrated by speed-vacuum drying (Rotational Vacuum Concentrator RVC 2-18, Germany). The residuals were re-suspended in an equal volume of specific culture medium.

2.4.3 MTT assay

The supernatant of the tested strain was determined for cytotoxicity against tumor cell lines including Caco-2 and HepG2 cells compared with Vero cells, as previously described [41, 42]. Briefly, all cell lines were proliferated at 37°C in a humidified atmosphere containing 5% CO_2 in specific culture medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (0.1 mg/ml). Caco-2 cells were proliferated in Dulbecco modified Eagle's minimal essential medium (DMEM) and Vero cells were proliferated in medium 199 (M199). The final density of each cell line as 1 x 10^5 cells per well was seeded in 96-well plates. Caco-2 and Vero cells were incubated at 37°C overnight before treatment with 10% v/v of the strain supernatant sample. All treated well plates were incubated at 37°C for 24 h in a 5% CO_2 incubator before adding the MTT solutions in each well and incubating at 37°C for 3 h in a 5% CO_2 incubator. The supernatant of each well plate was discarded before adding dimethyl sulfoxide (DMSO) and mixing gently. Optical density was monitored at 570 and 595 nm by a microplate reader. Cisplatin (50 and 25 µM) and MRS broth were used as positive and negative controls, respectively. The experiment was performed in triplicate with three independent assays. Percentage of cell viability was calculated using the equation below.

$$\text{Percentage of cell viability (\%)} = \frac{\text{Average absorbance of treated cells}}{\text{Average absorbance of negative control}} \times 100$$

2.5 Statistical analysis

Statistical analysis was conducted by one-way ANOVA following Tukey's method using SPSS version 22.0. A probability of $P < 0.05$ was considered to be significant.

3. Results and Discussion

3.1 Phenotypic and chemotaxonomic characterization

Strain FM11-1 was Gram-positive, facultatively anaerobic, non-motile, non spore-forming and spherical or ovoid at 0.8-1 µm in diameter and arranged in pairs or chains (Figure 1). Colonies on MRS agar plates were circular, low-convex, white with entire margins and non-pigmented (approximately 1 mm in diameter). Strain FM11-1 produced L-lactic acid from glucose homofermentative but did not produce gas from glucose. This strain produced diacetyl. Catalase activity, hydrolysis of arginine, nitrate reduction, slime production from sucrose and α-hemolysis were negative. Growth occurred at 10-42°C, pH 6.0-10.0 and within 8% (w/v) NaCl. Acid was produced from *N*-acetylglucosamine, ribose, aesculin, amygdalin, L-arabinose, arbutin, D-cellobiose, fructose, galactose, glucose, glycerol, D-lactose, mannose, mannitol, methyl-α D-manopyranoside, D-maltose, D-melibiose, salicin, sucrose, D-raffinose, D-tagatose and D-trehalose. The activities of acid phosphatase, esterase lipase (C8) and valine arylamidase were positive, while phosphohydrolase was weakly positive but leucine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosaminidase, α-glucosidase, α-mannosidase, α-fucosidase, esterase (C4), lipase (C14), alkaline phosphatase and trypsin had negative activities.

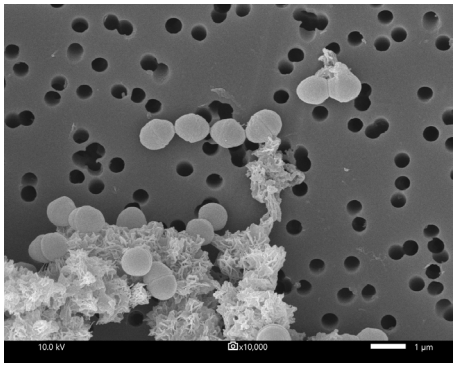

Figure 1. Scanning electron micrograph of strain FM11-1 grown in MRS broth at 37°C for 48 h (Bar, 1 µm)

Strain FM11-1 was differentiated from *E. lactis* LMG 25958^T, *E. durans* NBRC 100479^T, *E. faecium* NRIC 1145^T and *E. ratti* DSM 15687^T as previously reported [43-45] based on growth temperature and pH, enzyme activity and acid production from carbohydrates, as shown in Table 1.

Strain FM11-1 contained $C_{19:0}$ cyclo *ω*8c (37.9%) and $C_{16:0}$ (15.4%) as major cellular fatty acid components similar to *E. lactis* LMG 25958^T (43). Summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω*6c) (14.9%), summed feature 8 ($C_{18:1}$ *ω*7c or $C_{18:1}$ *ω*6c) (8.9%), $C_{18:0}$ *ω*9c (5.1%), $C_{20:0}$ (3.5%), $C_{18:0}$ (2.2%), iso-$C_{19:0}$ (1.16%), and $C_{14:0}$ (6.6%) were detected. This strain had no $C_{17:0}$ 2-OH and summed feature 7 and differed from *E. faecium* NRIC 1145^T, *E. durans* NBRC 100479^T and *E. ratti* DSM 15687^T (Table 2). These results showed that strains FM11-1 and *E. lactis* LMG 25958^T were clearly different from other species.

Table 1. Characteristics differentiating strain FM11-1 from related type strains

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	10-42	10-42	10-37	10-42	10-42
pH range for growth	6-10	5.5-10	5.5-10	5.5-10	5.5-10
API ZYM					
Alkaline phosphatase	-	-	-	-	+
β-Galactosidase	-	-	+	-	-
Lipase	-	-	+	+	-
Acid production from					
N-Acetylglucosamine	+	+	+	-	w
Amygdalin	+	w	-	-	w
Arbutin	+	w	+	w	w
Glycerol	+	-	+	-	-
Mannitol	+	+	+	+	w
D-Melibiose	+	-	-	-	-
Methyl-αD-manopyranoside	+	+	-	+	+
Methyl-αD-glucopyranoside	-	-	-	-	w
Potassium gluconate	-	-	-	-	w
Raffinose	+	w	-	w	-
Salicin	+	w	w	-	w
D-Trehalose	+	+	+	+	w

Strains: 1, FM11-1; 2, *E. lactis* LMG 25958^T; 3, *E. faecium* NRIC 1145^T; 4, *E.durans* NBRC 100479^T; 5, *E.ratti* DSM 15687^T and +, positive; w, weakly positive; -, negative reactions. All characteristics were determined in this study.

Table 2. Cellular fatty acid composition of strain FM11-1 and related type strains

Fatty acids	1	2	3	4	5
Saturated fatty acids					
$C_{9:0}$	-	-	-	-	2.5
$C_{14:0}$	6.6	5.9	5.7	7.8	4.2
$C_{15:0}$	-	-	-	1.3	-
$C_{16:0}$	**15.4**	**16.9**	**33.3**	**43.8**	**36.7**
$C_{18:0}$	2.2	2.0	1.8	2.8	3.1
$C_{20:0}$	3.5	1.9	-	1.1	-
Unsaturated fatty acids					
$C_{17:0}$ 2-OH	-	-	1.4	3.0	3.2
$C_{18:1}$ ω9c	5.1	3.8	9.0	**11.3**	**13.7**
$C_{19:0}$ cyclo ω8c	**37.9**	**42.6**	-	-	-
$C_{20:4}$ ω6,9,12,15c	-	-	-	-	1.6
Branched fatty acids					
iso-$C_{16:0}$	-	-	-	1.6	-
iso-$C_{19:0}$	1.2	1.3	1.4	3.3	1.3
Summed feature 3[a]	**14.9**	**12.4**	6.9	**14.7**	1.1
Summed feature 7[b]	-	-	**38.1**	-	**32.1**
Summed feature 8[c]	8.9	9.9	-	9.3	-

Strains: 1, FM11-1; 2, *E. lactis* LMG 25958^T; 3, *E. faecium* NRIC 1145^T; 4, *E. durans* NBRC 100479^T; and 5, *E. ratti* DSM 15687^T. All data are shown as percentage of total fatty acids and are determined in this study. -, Not detected.

Amounts of fatty acids ≤ 0.5% were not presented.

[a]$C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c, [b]$C_{19:1}$ ω7c and/or $C_{19:1}$ ω6c; $C_{19:0}$ cyclo ω10c and/or $C_{19:0}$ ω6, [c]$C_{18:1}$ ω7c or $C_{18:1}$ ω6c

3.2 Genotypic and genomic characterization

The 16S rRNA gene sequence indicated that strain FM11-1 belonged to genus *Enterococcus* and was closely related to *E. durans* NBRC 100479^T (99.7%), *E. faecium* NRIC 1145^T (99.6%), *E. hirae* ATCC 9790^T (99.4%), *E. lactis* LMG 25958^T (99.3%), *E. thailandicus* DSM 21767^T (99.1%), *E. mundtii* DSM 4838^T (98.9%), *E. canis* NBRC 100695^T (98.9%) and *E. ratti* DSM 15687^T (98.9%). The phylogenetic tree (Figure 2) showed that strain FM11-1 shared a cluster with *E. faecium* NRIC 1145^T and *E. lactis* LMG 25958^T as the closest relatives and formed a separate branch within the clade of the genus *Enterococcus*. However, from the results of 16S rRNA gene sequence similarity, it was difficult to identify strain FM11-1 as any *Enterococcus* species [12-14]. The draft genome sequence of strain FM11-1 (BKZS00000000) had 2,784,928 bp, which contained 2,586 coding sequences with *in silico* G+C content of 38.07 mol % in the range of genus *Enterococcus* [6]. The genomic features of the strain are presented in Table 3. The phylogenomic tree indicated that strain FM11-1 shared the same node with *E. lactis* LMG 25958^T and *E. faecium* NRIC 1145^T, while *E. durans* NBRC 100479^T and other species formed separate clusters (Figure 3). Strain FM11-1 exhibited the ANIb and ANIm values of draft genome of *E. lactis* LMG 25958^T (JADZMB000000000), with 97.23 and 98.30% similarity, which were higher than 95-96% needed for species delineation [32] but showed 93.93 and 94.87 % with *E. faecium* NRIC 1145^T (UFYJ01000001), as shown in Table 4. The digital DNA-DNA hybridization (dDDH) between the genomes of strain FM11-1 and the closest type strain, *E. lactis* LMG 25958^T was 83.7% (Table 4), which was higher than the cut-off value of 70% for species delineation [46], offering support that strain FM11-1 was *Enterococcus lactis* [15, 16]. Therefore, according to polyphasic characterization and genome analysis, this strain was identified as *Enterococcus lactis*.

Strain FM11-1 contained 22 genes involved in carbohydrate fermentation. Nine gene subsystems contributed to mixed-acid fermentation and 7 gene subsystems advocated lactate fermentation, including two genes coding for L-lactate dehydrogenase (L-LDH: EC 1.1.1.27) and one gene coding for D-lactate dehydrogenase (D-LDH: EC1.1.1.28). The strain also contained several specialty genes (91 genes) that were clustered by gene function. The functions included were antibiotic resistance: NDARO (National Database of Antibiotic-Resistant Organisms), antibiotic resistance: CARD (Comprehensive Antibiotic Resistance Database), antibiotic resistance: PATRIC (a bioinformatics resource that is designed to enable a comparative genomic analysis of bacterial pathogens and also provides information on genes that are linked to antibiotic resistance), Drug Target: TTD (Therapeutic Target Database, a database providing information about the known and explored therapeutic protein and nucleic acid targets, the targeted disease, pathway information and the corresponding drugs directed at each of these targets, Drug Target: DrugBank, Transporter: TCDB, Virulence Factor: PATRIC_VF, Virulence Factor: VFDB and Virulence Factor: Victors. Virulence and antibiotic resistance genes in *E. faecium* were also found in higher quantities than in *E. lactis* and strain FM11-1. However, enterococcal surface protein (*esp*), vancomycin (or other glycopeptides), histidine kinase (*vanF*), D-alanine-(R)-lactate ligase (*vanM*), D-alanyl-D-alanine dipeptidase of vancomycin resistance (*vanX*) and D-Ala-D-Ala dipeptidase/carboxypeptidase (*vanXY*) were absent in *E. lactis* and strain FM11-1. Specialty genes information differentiated *E. lactis* from *E. faecium* strain, as shown in Figure 4. The virulence *esp* gene is involved in mechanisms by which the cells adhere to biotic and abiotic surfaces and in biofilm formation [47], while *van* genes are antibiotic resistance genes involved in glycopeptide resistance and multidrug resistance, particularly vancomycin resistance [48]. These specialty genes (resistance and virulence factor genes) found in *Enterococcus* strains were significantly different between species.

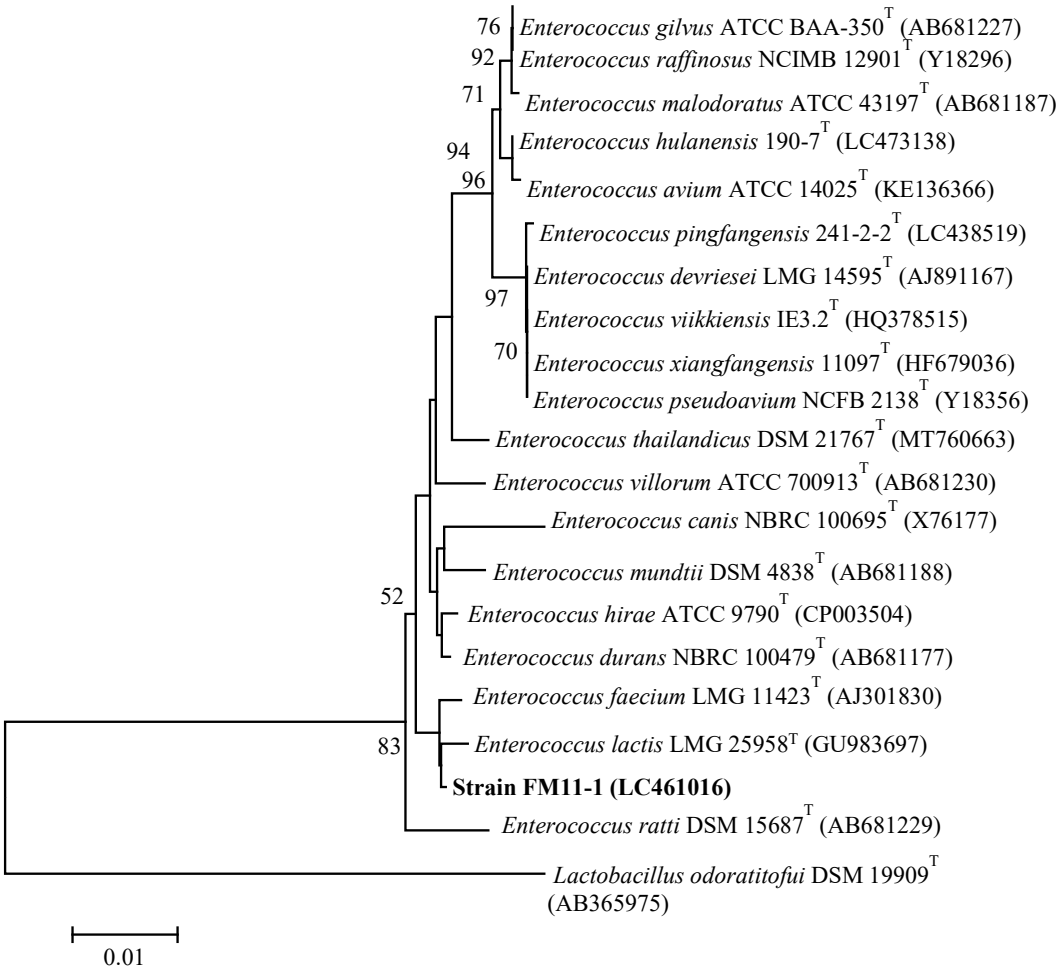

Figure 2. Neighbor-joining tree of strain FM11-1 and related *Enterococcus* species based on 16S rRNA gene sequences. Bootstrap percentages >50% based on 1000 replications are given at nodes. Bar, 0.01 substitutions per nucleotide position.

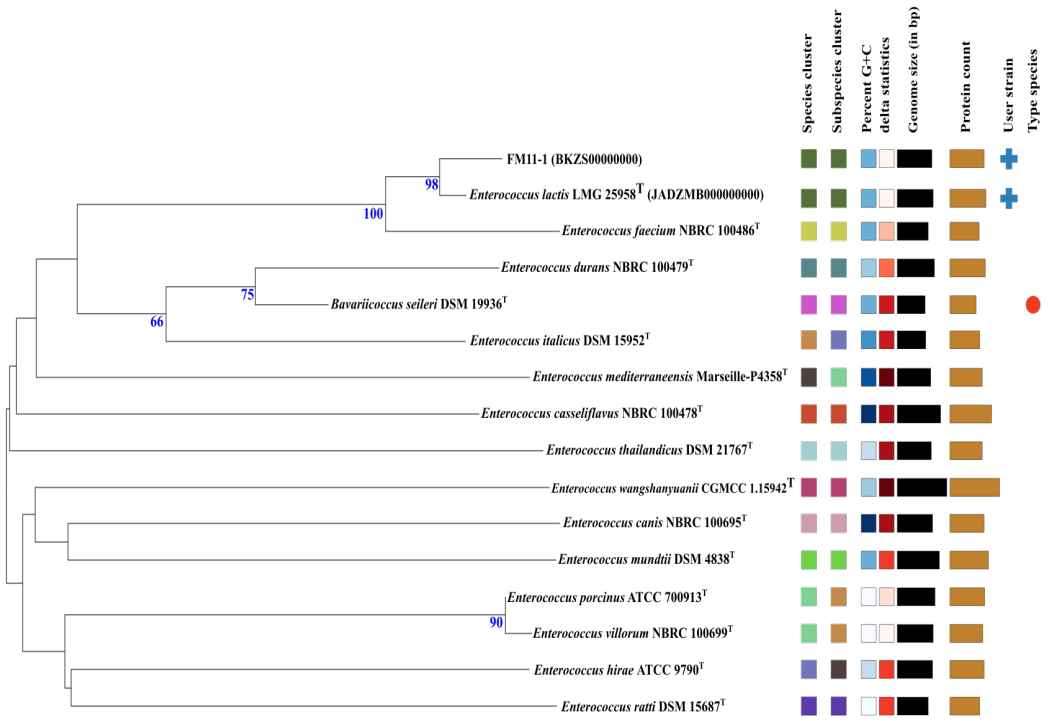

Figure 3. Phylogenomic tree based on TYGS result of strain FM11-1 with related type strains. Numbers on top of each branch are GBDP pseudo-bootstrap support values from 100 replications. Leaf labels are annotated by affiliation to species (1), subspecies clusters (2), genomic G+C content (3), δ values (4), genomic size (bp) (5), countable protein (6) and kind of strain (7). GenBank accession numbers are illustrated in parentheses; master record accessions are truncated.

Table 3. Genomic features of strains FM11-1 (BKZS00000000), *E. lactis* LMG 25958^T (JADZMB000000000), *E. faecium* NRIC 1145^T (UFYJ01000001), *E. durans* NBRC 100479^T (BCQB00000000) and *E. ratti* DSM 15687^T (JXLB01000000)

Attribute	FM11-1	LMG 25958^T	*NRIC 1145^T	*NBRC 100479^T	*DSM 15687^T
Genome size (bp)	2,784,928	2,915,113	2,667,210	3,017,302	2,485,659
G+C content (%)	38.07	38.1	38.1	37.8	34.3
Genome coverage	208x	395x	100x	146x	100x
N50	73,221	101,827	2,529,580	53,575	118,830
Number of contigs	112	236	7	122	78
No. of coding sequences	2,586	2,838	2,521	4,059	2,130
RNA genes	56	70	90	53	48

*Data obtained from GenBank

Table 4. ANIb and ANIm values (%) and *in silico* DNA-DNA hybridization (dDDH) of strain FM11-1 and its closest related type strains

Query genome	Reference genome	ANIb	ANIm	% dDDH*	Model C.I.	Distance	Prob. DDH ≥70%	G+C difference
1	2	97.23	98.30	83.7	80.9 – 86.2 %	0.0191	93.04	0.07
1	3	94.15	94.67	58.3	55.5 – 61 %	0.0547	46.47	0.03
1	4	78.13	86.68	24.6	22.3 – 27.1 %	0.177	0.01	0.28
1	5	76.21	84.58	21.4	19.2 – 23.9 %	0.2047	0	3.78

Draft genomes: 1, FM11-1 (BKZS00000000); 2, *E. lactis* LMG 25958^T (JADZMB000000000); 3, *E. faecium* NRIC 1145^T (UFYJ00000000); 4, *E. durans* NBRC 100479^T (BCQB00000000); and 5, *E. ratti* DSM 15687^T (JXLB01000000)

*Advised formula 2 (identities/HSP length), as liberated genome length that is prosperous against the utilization of partially draft genome.

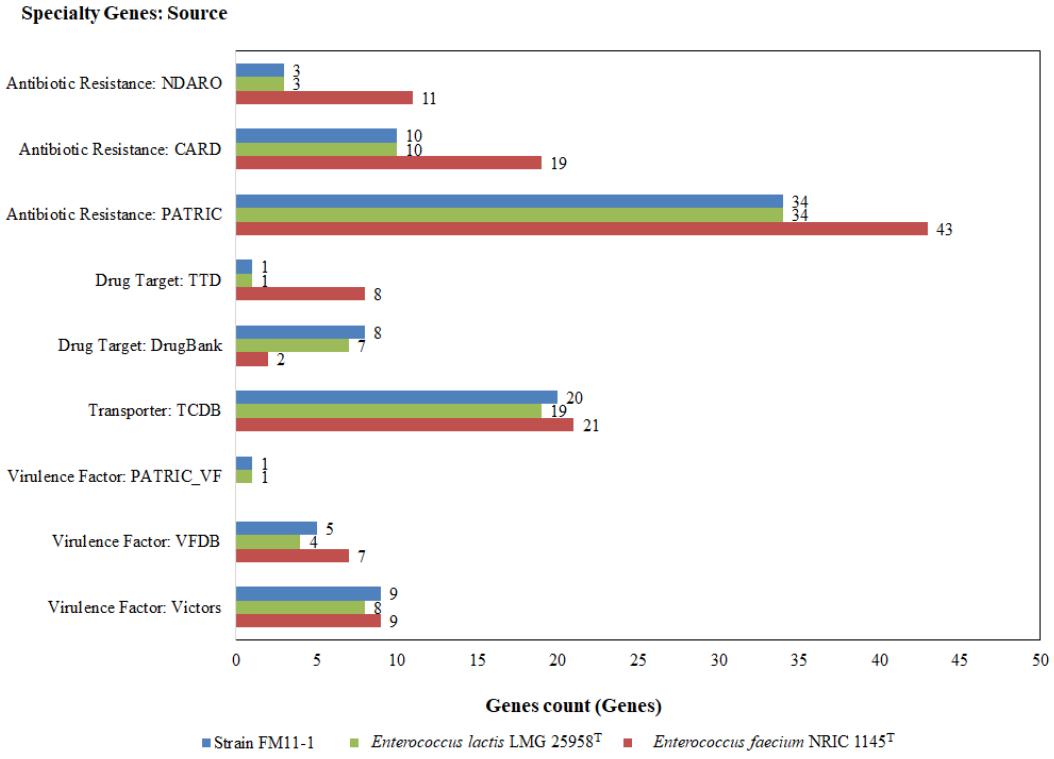

Figure 4. Number of specialty genes of strain FM11-1, *E. lactis* LMG 25958^T and *E. faecium* NRIC 1145^T

3.3 Screening of probiotic properties

Strain FM11-1, identified as *E. lactis*, adhered to Caco-2 cells (0.43 ± 0.11%) and its adhesion ability was not significantly different ($P = 0.547$) from *Lb. rhamnosus* GG (0.40 ± 0.05%). However, some other strains, *E. lactis* IITRHR1 [49] and *E. lactis* IW5 [50] adhered to intestinal epithelial cells and Caco-2 cells, respectively. High adhesive ability, which offers the host health benefits, encouraged the residence time of LAB strains in the gut, impeded pathogenic microorganisms and protected intestinal epithelial cells (IECs) by enhancing mucus synthesis and stimulating host defense peptide production [51]. The adhesive ability depended on several factors such as extracellular adhesins [52], bacterial cell surface-associated lipoteichoic acid and proteinaceous factors [53, 54] and passive entrapment of the bacterial cells by fimbrial cells [55]. FM11-1 also showed 95.27±0.01% anti-proliferative effect on Caco-2, 97.77±0.03% on HepG2 and 188.60±0.25% on Vero cells. Therefore, this strain had no anti-proliferative activity as reported by Thamacharoensuk *et al*. [56]. Similar to our study, *E. lactis* IW5 secretions exhibited no toxic effect on normal cells [51], and *E. lactis* IITRHR1 lysate did not inhibit the proliferation of hepatocytes [51]. Moreover, strain FM11-1 tolerated pH 2 and 0.3% bile, exhibited bile salt hydrolase activity and assimilated cholesterol, as reported previously [17]. Following FAO/WHO recommendations [57], desirable probiotics should include resistance to gastric acidity, bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, non-hemolysis activity, bile salt hydrolase activity and reduced serum cholesterol levels. However, further studies on *in vivo* animal models are required to elucidate the safety of this strain.

4. Conclusions

Strain FM11-1 was identified as *Enterococcus lactis* based on phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence similarity and draft genome analysis. Results of ANIb and digital DNA-DNA hybridization were 97.23% and 83.7%, respectively, compared to *E. lactis* LMG 25958^T. Strain FM11-1 showed adhesion ability on Caco-2 cells in the range of *Lb. rhamnosus* GG, a widely used probiotic strain, and did not express cytotoxic activity on Vero cells (normal cells).

5 Acknowledgements

This study was funded by a grant from the International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund (GCURP_58_01_33_01), Chulalongkorn University, as well as from Chulalongkorn University. We thank Dr. Sitanan Thitiprasert, Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, for analysis of the end products from glucose fermentation.

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