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Whole genome-based safety assessment of *Lacticaseibacillus paracasei* TISTR 2688, a potential probiotic for oral health care product

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Abstract

Lacticaseibacillus paracasei TISTR 2688, anti-oral pathogen activity possessing probiotic, was originally isolated from fermented termite comb. In this study, the safety of TISTR 2688 was investigated by whole genome analysis. The whole genome of TISTR 2688 was sequenced with two platforms, Illumina MiSeq and Oxford Nanopore Technologies (ONT). After hybrid assembly and annotation, it was shown that TISTR 2688 contained a singular chromosome of 3,209,739 bp with 46.40% GC content. Four plasmids, 57,771 bp, 65,854 bp, 7,511 bp and 6,877 bp were identified. The strain was confirmed to be *Lacticaseibacillus paracasei* with the average nucleotide identity (ANI) of 97.78% to the species' type strain ATCC 25302. TISTR 2688 did not harbor transferable antimicrobial resistance (AMR) genes, genes encoding bile salt hydrolase, and had no pathways for production of critically important antimicrobials for human medicine. Two copies of putative genes encoding D-lactate dehydrogenase were identified in the genome. Moreover, 9 prophage regions including 3 intact prophages, 3 incomplete and 3 questionable prophages were identified. None of the AMR genes were located on any plasmids or prophage regions. This finding together with the results of previous phenotypic assay and acute oral study indicated that *L. paracasei* TISTR 2688 was proven to be a safe probiotic candidate for human use.

Keywords: *Lacticaseibacillus Paracasei*; Probiotics; Safety Assessment; Whole Genome Sequencing

1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1]. Lactobacillus and Bifidobacterium are major genera of probiotics used globally [2]. Nowadays, probiotics have been incorporated into various products, including foods, functional foods, dietary supplements, beverages, etc. Health benefits generated by some probiotics were reviewed elsewhere [3, 4]. Recently, the possibility of probiotics in the field of oral health have been widely investigated. Several in vitro assays and clinical studies indicated the promising results of probiotics in prevention and treatment of dental caries and periodontal diseases [5-8].

L. paracasei TISTR 2688 was originally isolated from fermented termite comb, fermented liquid obtained from 10-days fermentation of termite comb mixed with cooked sticky rice and water from washing rice. This bacterium was deposited in TISTR culture collection under registration number TISTR 2688. The strain TISTR 2688 exhibited probiotic properties e.g. resistance to simulated gastric and simulated small intestinal juices and good adherence to the Caco-2

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and HT-29 human colon cell lines [9]. Ruengsomwong et al. [10] demonstrated that TISTR 2688 was resistant to 1 mg/mL lysozyme, had high inhibitory activity against oral pathogenic bacteria including *Actinomyces vericosus*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* subsp. *polymorphum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Streptococcus mutans*, *S. sanguinis*, and *S. sobrinus*. In addition, TISTR 2688 could inhibit *S. mutans* biofilm formation on prosthetic teeth. The results implied the potential use of TISTR 2688 in an oral health care product. FAO/WHO [11] and EFSA, 2021 [12] suggested that the safety of probiotic intentionally used in human had to be thoroughly characterized by phenotypic testing and whole genome sequencing. So far, TISTR 2688 was phenotypically proven to be non-hemolytic and susceptible to ampicillin, chloramphenicol, clindamycin, erythromycin, gentamycin, kanamycin, streptomycin and tetracycline. In addition, acute oral toxicity assay in rats at dose of 1×10^{10} CFU/kg body weight showed no mortality, no toxicity or evidence of gross pathological alterations [10]. No whole genome sequences has been characterized yet. To obtain more information essential for safety evaluation, whole genome sequences of TISTR 2688 was further analyzed in this study.

2. Materials and Methods

2.1. Bacterium and growth conditions

L. paracasei KT-5 TISTR 2688 kept in 40% glycerol at -80 °C was grown in de Man, Rogosa and Sharpe (MRS) medium (Merck, Darmstadt, Germany) pH 6.8 supplemented with 0.05% L-cysteine HCl (Merck, Germany). Incubation was carried out anaerobically in an anaerobic jar (Thermo Scientific™, Lenexa KS, USA) containing an AnaeroPack (MCG, Tokyo, Japan) at 37 °C for 24–48 hours.

2.2. Safety assessment using whole genome sequences

2.2.1. Extraction of total DNA

TISTR 2688 was grown in MRS broth pH 6.8 containing 0.2% glycine (w/v) and incubated under anaerobic conditions at 37 °C for 6-10 hours. Cell pellets were harvested by centrifugation at 6,500 ×g for 5 min at 4 °C and washed once with normal saline. The washed pellets were used for genomic DNA extraction using Wizard Genomic DNA Purification Kit (Promega Corporation, WI, USA) with modified lysis buffer [13]. The modified lysis buffer contained 10 mg/ml lysozyme (Pharmacia Biotech, USA) and 25 U/ml mutanolysin (Sigma-Aldrich, USA) in 50 mM EDTA pH 8. The quality and concentration of the extracted genomic DNA were measured using BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan). High-purity genomic DNA with an OD260/OD280 ratio of 1.8–2.0 and an OD260/OD230 ratio of 2.0–2.2 was used for whole-genome sequencing.

Plasmid DNA was extracted using QIAprep Spin Miniprep kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. The number of plasmids and their size were visualized using 0.5% agarose gel electrophoresis in 0.5X TBE buffer.

2.2.2. Whole genome sequencing and genome assembly

Long read sequencing was performed at Novogene AIT Genomic Singapore Pte., (Singapore) using Oxford Nanopore Technologies (ONT) (Rapid sequencing kit, MinIONTM device, Oxford Nanopore Technologies, UK) while short read sequencing was run by Omics Science and Bioinformatic Center (Thailand) on Illumina Mi-Seq paired-end (250x2) (llumina, Inc., USA). For nanopore reads, the ONT adaptors were trimmed using Porechop v0.2.4 (https://github.com/rrwick/Porechop). Quality control of ONT reads was undertaken using NanoPlot v1.28.1 and NanoFilt v2.5.0 to eliminate short (< 500 bases) and low-quality reads (a mean quality score < 9) [14]. For Illumina reads, the Fastp v0.20.1 was used for quality filtering and adapter trimming. The quality of the processed reads was assessed with the FastQC tool, version 0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The good-quality reads were assembled by the hybrid assembly mode of Unicycler v0.4.8 [15]. The genome was quality-checked using default settings in QUAST, version 5.0.2 [16]. The gene prediction and computational annotation of protein-coding genes were performed using DFAST v1.2.6 [17], a software tool for the rapid annotation of prokaryotic genomes.

2.2.3. Species identification

The presumptive species identification was done using 16S rRNA gene sequences. The sequences from all 16S rRNA gene copies were compared to each other using Clustal Omega multiple sequence alignment tool [18]. The similarities of the 16S rRNA genes were compared to the NCBI's 16S ribosomal RNA from curated type strain sequences from bacteria and archaea database. The average nucleotide identity (ANI) to the type strains of the presumptive and closely

related species were identified using JSpeciesWS [19]. The ANI value above 95% was used as the criterion for species identification.

2.2.4. Identification of genes coding for virulence factor, toxins and undesirable metabolites

Genes related to known bacterial toxins, the production of biogenic amines, and D-lactic acid were identified using the KEGG database [20].

2.2.5. Identification of antimicrobial resistance genes.

Genes related to known antimicrobial resistance (AMR) were identified using the Comprehensive Antibiotic Resistance Database (CARD) [21] using the Resistance Gene Identifier (RGI) to predict resistome(s) based on homology and SNP models with "perfect and strict hits only" option. The additional database used was the ResFinder database (https://cge.cbs.dtu.dk/services/ ResFinder/) using ResFinder 4.1 software [22]. The search parameters for the analysis were sequence identity 90% and coverage 60%. Association of the AMR genes with two mobile elements including plasmids and prophages were identified. The existence and location of prophages in the genome were identified using the Phage Search Tool Enhanced Release PHASTER [23].

2.2.6. Identification of antimicrobial production

The WHO critically important antimicrobials (WHO-CIA list) [24] was used as a reference for the antimicrobial of concern. Since there is no database available for the identification of the genes involved in the biosynthesis of all antimicrobials in the list, the KEGG database is currently used as the best resource for this purpose [13].

2.3. Safety assessment using phenotypic method

2.3.1. Determination of D-lactic acid L-lactic acid

TISTR 2688 was anaerobically grown in MRS broth at 37 °C for 14-16 hours. The overnight culture was centrifuged at 4,000 ×g for 10 min. The supernatant was collected, filter sterilized and used for quantifying L- and D- lactate using D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit according to manufacturer protocol (Megazyme, Bray, Ireland).

2.3.2. Detection of bile salt hydrolase (*BSH*) *using plate assay*

Single colonies of TISTR 2688 anaerobically grown on MRS agar at 37 °C for 18 hours were streaked on MRS agar containing 0.5% (w/v) taurodeoxycholic acid (Sigma Aldrich, USA). Incubation was done anaerobically for 5 days at 37 °C. *Lactobacillus acidophilus* ATCC 4356, a BSH producing strain, was used as a positive control. After incubation, the deconjugation activity was observed as previously described [25]. The presence of either the precipitated halos around the colonies or the white precipitates within the colonies when compared to the MRS control indicated BSH activity.

3. Results

In this study, the safety of *L. paracasei* TISTR 2688 was evaluated at the whole genome level by searching for antibiotic resistance genes, virulence- and toxin genes.

3.1. Safety characteristics of TISTR 2688

3.1.1. Genome characteristics of TISTR 2688

By combining whole genome sequencing information obtained from Illumina MiSeq™ and ONT platforms, the complete genome of TISTR 2688 was shown to contain a single circular chromosome of 3071726 bp with four plasmids of 57771 bp, 65854 bp, 7511 bp and ,877 bp, respectively. The genome size was 3209,739 bp with GC content of 46.4%, 3111 coding sequences (CDS), 15rRNA genes and 60 tRNA genes. No CRISPRs genes was detected (Table 1).

Table 1 Main characteristics of TISTR 2688 genome

Genome size (bp)	**3209739**
Chromosome size (bp)	3071726
Size of plasmid 1 (bp)	57771
Size of plasmid 2 (bp)	65854

Size of plasmid 3 (bp)	7511
Size of plasmid 4 (bp)	6877
GC content (%)	46.4
Number of CDSs	3111
Number of rRNAs	15
Number of tRNAs	60
Number of CRISPRs	0

3.1.2. Species identification

TISTR 2688 contained five copies of 16S rRNA gene with 99.68-100% identity to each other. The full 16S rRNA genes (1,566 nt) exhibited the highest similarity (99.80-99.87%) to those of *Lacticaseibacillus paracasei* R094 (JCM 8130T). The strain also exhibited the highest ANI values (>97%) to both subspecies of *Lacticaseibacillus paracasei* type strains (Table 2 and 3).

Table 2 Average nucleotide identity (ANI) based on BLAST (ANIb) of TISTR 2688

Genome	**ANIb [%]**	**Aligned [%]**	**Aligned [bp]**	**Total [bp]**
L. paracasei subsp. *paracasei* ATCC 25302[T]	97.78	78.25	2511744	3209739
L. paracasei subsp. *tolerans* NBRC 15906[T]	97.26	64.69	2076276	3209739
L. casei DSM 20011 [T]	77.90	54.97	1764529	3209739
L. zeae DSM 20178 [T]	77.79	56.59	1816394	3209739
L. chiayiensis NCYUAS[T]	77.11	54.85	1760542	3209739
L. rhamnosus DSM 20021 [T]	76.87	57.70	1852014	3209739

Table 3 Average nucleotide identity (ANI) based on MUMmer(ANIm) of TISTR 2688

Genome	**ANIm [%]**	**Aligned [%]**	**Aligned [bp]**	**Total [bp]**
L. paracasei subsp. *paracasei* ATCC 25302[T]	98.60	80.48	2583159	3209739
L. paracasei subsp. *tolerans* NBRC 15906[T]	98.38	68.43	2196528	3209739
L. casei DSM 20011 [T]	84.73	20.01	642201	3209739
L. zeae DSM 20178 [T]	84.71	19.72	632965	3209739
L. chiayiensis NCYUAS [T]	84.35	17.17	551143	3209739
L. rhamnosus DSM 20021 [T]	84.09	16.45	528008	3209739

With the 95% ANI cut-off threshold, the strain was concluded to belong to the species *Lacticaseibacillus paracasei*.

3.1.3. Identification of genes of safety concern

Toxin encoding genes

The genome of TISTR 2688 contained two genes identified as pore-forming bacterial toxins, *tlyC* and *hlyIII* (Table 4). Both genes were widespread in Lactobacillus, including strains with a history of safe use in the food industry and probiotics such as *L. plantarum* 299V and *L. plantarum* PMO 08 in kimchi, among others [26]. Previous hemolysis assay

indicated that TISTR 2688 had no hemolytic activity [10]. Hence, the presence of these two genes in TISTR 2688 genome was not of safety concern.

Table 4 Predicted toxin encoding genes in TISTR 2688 genome

KO	Detail	Gene ID
K03699	*tlyC*; magnesium and cobalt exporter, CNNM family	MGA_1016, MGA_1986
K11068	*hlyIII*; hemolysin III	MGA_892
K11041	eta; exfoliative toxin A/B	MGA_2608

The genome of TISTR 2688 also contained *efa* gene encoding exfoliative toxin (ETs). ETs are serine proteases involved in the pathogenesis of *S. aureus* skin infections such as scalded skin syndrome (SSSS) in infants and young children [27]. To the best of our knowledge, there have been no case report on SSSS causing by *L. paracasei* so far. This implied that *efa* gene might not play a role as a virulence factor in this case. However, the actual function of *efa* gene in TISTR 2688 should be further investigated.

3.1.4. Genes related to biogenic amines production

The genome contained a gene encoding ornithine decarboxylase, indicating the ability of TISTR 2688 to produce putrescine from ornithine (Table 5).

Table 5 Identified putative genes for biogenic amine production in TISTR 2688 genome

EC No.	Name	Functions	Gene ID
4.1.1.18	Lysine decarboxylase	production of cadaverine	ND
4.1.1.116	D-ornithine/D-lysine decarboxylase	production of cadaverine	ND
4.1.1.19	Arginine decarboxylase	Arginine-->agmatine	ND
3.5.3.11	Agmatinase	Agmatine-->putrescine	ND
2.5.1.16	Spermidine synthase	Putrescine-->spermidine	ND
2.5.1.22	Spermine synthase	Spermidine-->spermine	ND
3.5.3.1	Arginase	Arginine-->ornithine	ND
4.1.1.17	Ornithine decarboxylase	Ornithine-->putrescine	MGA_1714
4.1.1.22	Histidine decarboxylase	Histidine--> histamine	ND
4.1.1.25	Tyrosine decarboxylase	Tyrosine--> tyramine	ND
4.1.1.28	Tryptophan decarboxylase	Tryptophan-->tryptamine	ND

As known, production of biogenic amines required both decarboxylase and transporter system. Therefore, further search for genes involved in putrescine transport system in TISTR 2688 genome was carried out. The search results revealed 4 genes, e.g. *pot*A, *pot*B, *pot*C, and *pot*D which code for PotA (spermidine/putrescine transport system ATP-binding protein; Gene ID MGA_949), PotB (spermidine/putrescine transport system permease protein; Gene ID MGA_950), PotC (spermidine/putrescine transport system permease protein; MGA_951) and PotD (spermidine/putrescine transport system substrate-binding protein; MGA_952), respectively. These proteins were reported to be the components of a spermidine/putrescine ABCtransporter system responsible for ornithine uptake in lactobacilli [28]. It should be noted that no *pot*E gene encoding putrescine-ornithine antiporter was identified on the genome of TISTR 2688. Since putrescine-ornithine antiporter was necessary for putrescine excretion [29], this implied that even the putrescine was produced, it could not be exported outside the bacterial cells.

This finding was in line with the characteristic of the *L. paracasei* ssp. *paracasei* strain F-19, a strain harboring gene encoding ornithine decarboxylase but not accompanying by a transporter required for putrescine generation. It should be emphasized that *L. paracasei* ssp. *paracasei* strain F-19 has been given generally-recognized-as-safe (GRAS) status (GRN Notice 840).

Putrescine and other polyamines in human body are generated by endogenous biosynthesis in cells, food intake and production by intestinal microbiota. The largest amount of putrescine in human are from food intake [30]. Occurrence of putrescine in food has several adverse effects on consumers's health. High amount of putrescine caused food poisoning. It could enhance toxic effects of histamine and tyramine as well. In addition, putrescine was a precursor for carcinogenic N-nitrosamines formation [31]. On the contrary, low concentration of intestinal polyamines, especially spermidine and putrescine produced by intestinal microbiota could be one of the important factors in the onset of intractable adult-type atopic dermatitis [32] and aging diseases [33]. Interestingly, there were reports showing that putrescine producing *Bifidobacterium animalis* subsp. *lactis* LKM512 could promoted longevity in 10-month-old Crj:CD-1 female mice by suppressing chronic low-grade inflammation [34] and improved cognitive flexibility in 10 weeks old Male C57BL/6 mice [35]. Recently, it was demonstrated that Edam cheese containing putrescine producing *L. plantarum* Inducia was harmless to mice and volunteers. All NHL-line mice fed with Edam cheese containing $2x10^8$ CFU/g of putrescine producing *L. plantarum* Inducia for 30 days showed no death and any adverse effects while healthy volunteers who consumed Edam cheese containing *L. plantarum* Inducia doses of $3x10^9$ CFU per serving for 3 weeks were in good health without discomfort or other negative symptoms [36]. In addition, Edam cheese containing *L. plantarum* Inducia could enhance the innate immunity markers of intestinal tract and blood. Up to the present, case reports caused by putrescine-producing bacteria have been barely found. Hence, the presence of ornithine decarboxylase on TISTR 2688 genome may not be a safety concern.

3.1.5. D-Lactic acid encoding genes

TISTR 2688 harbored two copies of putative gene encoding D-lactate dehydrogenase indicating the ability of TISTR 2688 to produce D-lactic acid from pyruvate (Table 6). Quantification of D- and L- lactic acid in supernatant using assay kit revealed that TISTR 2688 produced mainly D-lactate (92.87% D-lactate and 7.08% L-lactate). Certain probiotic bacteria, which have been given GRAS status, used in food and infant formula are capable of producing mixture of D- and L-lactic acid with variable ratios of the two isomers. These were *L. plantarum* LPwL ® (GRN Notice 847), *L. plantarum* Lp-115 (GRN Notice 722), *L. plantarum* 299V (GRN Notice 685). Moreover, two commercially available probioitcs used in food supplement were proven to be D- lactate producing strains, e.g. *Limosilactobacillus reuteri* DSM 17938 in Reufor Italchimici S.p.A, Italy and *L. rhamnosus* ATCC 53103 in Dicofor AG Pharma S.r.l, Italy [37]. It was reported that *L. bulgaricus* Lb-87 (Danisco) produced 97.6% D-lactate and 2.4% L-lactate while *L. plantarum* 299v produced 61.9% D-lactate.

Table 6 Putative genes involved in lactic acids production in TISTR 2688 genome

EC No.	Enzyme name	Function	Gene ID
1.1.1.28	D-lactate dehydrogenase	Pyruvate <--> D-lactate	MGA_151, MGA_2125

Usually, D- and L- lactic acid can be generated in human body by human tissues, fermentation of unabsorbed carbohydrates by intestinal microbiota and consumption of food containing D- lactate [38]. Under normal condition, D-form is produced in insignificant level and harmless. Nevertheless, increase in production of intestinal D-lactate can lead to the accumulation of D- lactate in bloodstream which in turn causing D-lactic acidosis especially in infant [39], children [40] and elders [38] with short bowel syndrome. Historically, it had been suggested that consumption of D-lactic producing bacteria were responsible for induction of D-lactic acidosis [41-43]. Except the patients with SBS, no case of D- lactate acidosis have been reported in healthy individuals yet. A systemic review of five randomized controlled trials from 2005 to 2017 covering 544 healthy infants exhibited that D- lactate producing probiotics and fermented infant formulas did not cause D-lactic acidosis in healthy children [44]. In addition, administering D-lactic producing probiotic bacteria were safe and did not cause any long-term increases in blood D -Lactic acid. Therefore, the ability in producing D-lactic acid of TISTR 2688 should not be considered a safety concern for the general population. Nevertheless, precautions should be taken for individuals with SBS.

3.1.6. Antimicrobial resistance genes.

The Antimicrobial resistance determinants in genome of TISTR 2688 were searched against CARD database with "perfect and strict hits only" option. Only one antibiotic resistance gene encoding small multidrug resistance (SMR)

antibiotic efflux pump was identified (Table 7). This gene was found to be located on the chromosome. Antibiotic susceptibility profile obtained from our previous study revealed that TISTR 2688 was susceptible to ampicillin, chloramphenicol, vancomycin, tetracycline, erythromycin, gentamicin, kanamycin, streptomycin and clindamycin. Hence, the functions of SMR antibiotic efflux pump found on the chromosome of on TISTR 2688 might be other than antibiotic resistance.

Table 7 Antimicrobial resistance genes identified by CARD

Gene ID	Position	Drug Class	Resistance mechanism	AMR gene family
MGA-2831	2931545-2931865	disinfecting agents and antiseptics	Antibiotic efflux	small multidrug resistance (SMR) antibiotic efflux pump

No acquired AMR genes were identified when searching by Res Finder 4.1 (with the default settings: 90% threshold and 60% minimum length).

Table 8 Prophage regions in TISTR 2688 genome identified by PHASTER

Region	Completeness	Score	No. Total proteins	Region Position
Chromosome				
1	questionable	70	53	864358-903178
2	incomplete	60	50	1141390-1177767
3	Intact	140	48	1218571-1253426
4	questionable	70	10	1353172-1360113
5	incomplete	30	19	1507638-1528717
6	Intact	100	18	2515281-2529020
7	incomplete	60	19	3023841-3037713
plasmid_1				
8	questionable	90	27	445-26813
plasmid_2				
9	Intact	100	22	25362-40720

Searching for prophage sequences in TISTR 2688 genome using PHASTER revealed nine prophage regions, including three questionable and six incomplete prophages (Table 8). Since none of AMR genes were found to locate on any plasmids or prophage regions, horizontal transfer of these putative AMR genes seemed unlikely to happen. As a consequence, TISTR 2688 was not a safety concern in term of antimicrobial resistance.

3.1.7. Genes associated with antimicrobial production

The WHO list of critically important antimicrobials for human medicine (WHO-CIA list) was used as a reference for the antimicrobials of concern. Since there has been no database available for the identification of the genes involved in the biosynthesis of all antimicrobials in the list, the KEGG database was currently used as the best resource for this purpose [13]. The search found no complete pathway for the production of the antimicrobials of concern (Table 9).

3.1.8. Genes involved in bile salt deconjugation

No gene encoding choloylglycine hydrolase (synonyms: bile salt hydrolase, BSH) was found in the genome of TISTR 2688.

Screening of BSH activity using plate assay by streaking TISTR 2688 on MRS agar containing 0.5% (w/v) TDCA plate confirmed that this strain did not possess BSH activity (Fig.1)

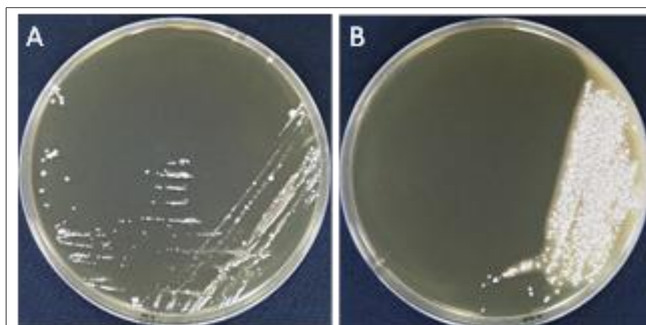

Figure 1 Detection of bile salt hydrolase (BSH) using plate assay. Colonies of TISTR 2688 (A) and *L. acidophilus* ATCC 4356 (B) were streaked on MRS agar containing 0.5% (w/v) taurodeoxycholic acid. The plates were incubated at 37°C under anaerobic conditions for 5 days

Table 9 Genes associated with antimicrobial production in TISTR 2688 genome, identified by KEGG

Map_ID	**Map_Name**	**Products**	**Results (no. of genes)**
Metabolism of terpenoids and polyketides			
map01052	Type I polyketide structures	several antibiotics including erythromycin, oleandomycin, tylosin, rifamycin	none
map00522	Biosynthesis of 12-, 14- and 16-membered macrolides	several antibiotics including erythromycin, oleandomycin, tylosin	none
map01051	Biosynthesis of ansamycins	rifamycin	none
map00253	Tetracycline biosynthesis	tetracyclin	none
map01054	Nonribosomal peptide structures	several antibiotics including Bacitracin, Pristinamycin IA	incomplete (1)
map01055	Biosynthesis of vancomycin group antibiotics	vancomycin	none
Biosynthesis of other secondary metabolites			
map00311	Penicillin and cephalosporin biosynthesis	penicillin, cephalosporin	incomplete (1)
map00332	*Carbapenem biosynthesis*	carbapenem antibiotics	incomplete (2)
map00261	*Monobactam biosynthesis*	monobactam antibiotics	incomplete (4)
map00331	*Clavulanic acid biosynthesis*	cluvulanic acid	none
map00521	*Streptomycin biosynthesis*	streptomycin	incomplete (4)
map00524	*Neomycin, kanamycin and gentamicin biosynthesis*	Neomycin, kanamycin and gentamicin	incomplete (1)

4. Conclusion

Safety evaluation of *L. paracasei* TISTR 2688 based on whole genome analysis exhibits that TISTR 2688 does not carry genes encoding deleterious toxins and bile salt hydrolase. TISTR 2688 is not capable of synthesizing critically important antimicrobial drugs for human medicine because no complete pathway for production of the antimicrobials of concern

is detected. The presence of genes encoding D-lactate dehydrogenase and ornithine decarboxylase in TISTR 2688 genome is not considered a safety concern. The strain harbored none of acquired antimicrobial resistance genes and has low risk of AMR gene transfers. Altogether, *L. paracasei* TISTR 2688 seems to be safe for healthy individuals. This works provided useful information which can be used as the supporting documents for registration of probiotic and probiotic products for human use.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest

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