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Full Paper

Genome-based assessment of safety characteristics of *Lacticaseibacillus paracasei* NY1301 and genomic differences in closely related strains marketed as probiotics

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The probiotic attributes of *Lacticaseibacillus paracasei* NY1301 were comprehensively characterized, and a comparison between the closely related LcA (Actimel) and LcY (Yakult) probiotic strains was conducted using genomic tools. All strains exhibited high genetic similarity and likely shared a common ancestor; differences were primarily expressed as minor chromosomal re-arrangements, substitutions, insertions, and deletions. Compared with LcY, NY1301 exhibited 125 single-nucleotide polymorphisms. NY1301 lacked virulence factors, antibiotic resistance genes, and mutations associated with antibiotic resistance and had a 46-kbp prophage. This prophage is spontaneously induced at low levels and remains in a non-lytic state under standard culture conditions. The observed causal adaptive mutations were likely related to niche adaptation within the respective laboratory or manufacturing processes that occurred during the maintenance of the strains. However, the phenotypic effects of these genomic differences remain unclear. To validate the safety of NY1301, we conducted an open-label trial with healthy participants who consumed excessive amounts of NY1301 (3.0×10^{11} cfu) daily for 28 days. The results of this trial and those of other *in vivo* studies, coupled with the long history of human consumption without established risks to humans, provide strong evidence confirming the safety of NY1301.

Key words: comparative genome, *Lacticaseibacillus paracasei*, safety assessment, probiotics, prophage

INTRODUCTION

Nissin York Co., Ltd. introduced a fermented milk-based beverage containing the probiotic strain *Lacticaseibacillus paracasei* NY1301, originally isolated from a human, into the Japanese market [1]. Since 1993, this strain has been cultivated in a blend of skimmed milk powder, sugar, and glucose, and the resulting mixture has been commercialized as a probiotic milk beverage known as Pilkul. Over the years, Pilkul has become a long-standing product with various line extensions offering different flavors and fruit juices, along with several novel functionalities [2–5]. Notably, the interaction between sugar and amino acids during the production of Pilkul induces Maillard-type reactions, giving the beverage a light coffee-brown color [6]. It is a popular Yakult-type product in Japan [1]. Although several Yakult-type products containing probiotic *L. paracasei* strains are available in Japan, their genomes have not been thoroughly examined [1], and the precise mechanisms underlying their functions remain to be elucidated. In this context, our study aimed to conduct a comprehensive analysis of the whole-genome sequence of NY1301 to investigate its safety traits at the genomic level. Additionally, we compared the genome structure and genetic variations of NY1301 with those of closely related strains that are well-known probiotics available in the market. Lastly, an open-label trial was conducted to assess the safety of consuming excessive amounts of NY1301.

MATERIALS AND METHODS

The NY1301 strain, which is used in our regular production processes, was obtained from the culture collection of Nissin York (Saitama, Japan). The genome sequence of NY1301 was generated using an Illumina HiSeq 2500 platform at Hokkaido System Science Co., Ltd. (Hokkaido, Japan). The genomic DNA was fragmented, and a sequence library was prepared using a TruSeq DNA PCR-Free Library Prep Kit (Illumina, San Diego, CA, USA). This library was sequenced by 101-bp paired-end sequencing. A total of 22,967,190 paired-end reads were obtained from the sequencing process, with a genome coverage of approximately 1,500×, and used for *de novo* assembly using

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the Velvet [7] software. Gap closing analysis was performed using the Platanus [8] software. Scaffolding was subsequently completed using the MUMmer [9] software and in-house scripts, aligning with the reference genome of *L. paracasei* TCS (CP038153.1, CP038154.1). The genome sequences of the following other *L. paracasei* strains were obtained from GenBank: LcA (CM001861.1, CM001862.1) from Actimel, LcY (CM001848.2, CM002348.1) from Yakult containing a single chromosome and plasmid with undefined nucleotides, and *L. paracasei* BL23 (FM177140.1). Subsequent annotation of all genomes was performed using the DFAST [10] software. To assess genetic relatedness among strains, we conducted average nucleotide identity (ANI) comparisons using the Kostas Lab web server [11]. The alignment of chromosomes was generated through pairwise blastn comparisons, and the identification of single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) in the genomic sequences was accomplished using the MUMmer software [9] with default settings. The results were visualized using the GenomeMatcher software [12]. To investigate the presence of virulence factors and toxin genes in NY1301, we searched VFDB [13]. Additionally, we investigated the genetic determinants associated with antimicrobial resistance in the genome using the CARD, RGI [14], and ResFinder [15] databases with predefined criteria (>85% identity and >80% coverage), as previously reported [16, 17]. To identify potential prophages within the genome, we used the PHASTEST tool [18].

In addition to the genome-based safety assessment, we conducted an open-label trial to investigate the safety of consumption of large amounts of NY1301, with the support of Medical Station Clinic (Tokyo, Japan). The study protocol was reviewed and approved by the Institutional Review Board of Aisei Hospital Ueno Clinic and adhered to the Principles of the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research (UMIN ID: 000036151). Eligible male and female participants, aged between 20 and 64 years, were recruited for the study. A total of 20 participants met the eligibility criteria and were selected to receive NY1301 treatment, which included consuming 3.0×10^{11} cfu daily for four weeks (Tables 1 and 2). Throughout the trial period, the participants recorded their physical condition, use of medications, and frequency of NY1301 ingestion. Clinical assessments were conducted during weeks 2 and 4 of the intake period, as well as at 2 weeks after the conclusion of the intake period. To ensure the accuracy and integrity of the clinical trial, EP Mediate Co., Ltd. (Tokyo, Japan) actively supported the study as a contract research organization.

RESULTS

Genome sequencing of NY1301 was performed first, followed by a comprehensive comparative analysis with the well-established genomes of the LcA and LcY strains from Actimel and Yakult, respectively [19, 20]. Our findings revealed that NY1301 and the LcA and LcY strains exhibited remarkably high levels of genomic homology, with an ANI of ≥99.99%, indicating close genomic relatedness. The genome size of NY1301 was similar to that of LcA but was 34 kb larger than that of LcY, implying greater genomic heterogeneity in NY1301 than in LcY (Table 3). Notably, the genomes of these strains displayed considerable synteny, with only one region of the LcY chromosome showing substantial disruption (Fig. 1a). This region mainly consists of a genomic island encompassing prophages and transposases, comprising 66 genes unique to NY1301. Further examination of genomic variations revealed the presence of minor InDels and 125 defined SNPs on the NY1301 chromosome, which served as differentiating factors between NY1301 and LcY (Fig. 1b).

On the NY1301 chromosome, we identified six putative prophages designated as regions 1–6 (Fig. 1a). Protein predictions revealed that most of the proteins in these regions shared high amino acid identity scores with known PLE1–PLE6 prophages

Table 1. Composition of the test beverage

Nutritional facts (value for daily dose, 300 mL)	
Energy (kcal)	135
Protein (g)	3.6
Fat (g)	0.0
Carbohydrate (g)	29.7
Sodium (mg)	45

The test beverages included milk fermented with NY1301 at more than 3.0×10^{11} cfu per three bottles of 100-mL each during the intervention. The beverages were distributed and stored at 0 to 10°C.

Table 2. Background of the participants

Parameter	Observed value
Age (years)	43.9 ± 14.2
Sex (male/female)	10/10
Height (cm)	166.68 ± 8.78
Body weight (kg)	59.67 ± 10.06
BMI	21.32 ± 1.99

Values indicate means ± standard deviation.
BMI: body mass index.

Table 3. General genomic features of NY1301 and the closely related *L. paracasei* strains

	NY1301	LcY	LcA
Chromosome size (bp)	3,076,977	3,042,653	3,068,555
GC content (%)	46.3	46.4	46.3
Protein-coding gene	2,992	2,937	3,003
rRNA gene	15	15	14
tRNA gene	60	59	62
Prophage clusters	6	5	6
Plasmid	1 (66,8 kb)	1 (59,6 kb)	1 (59,6 kb)

bp: base pairs.

in *L. paracasei* BL23 [21, 22]. Interestingly, region 4 is absent in LcY (Fig. 1a). Most phage proteins in NY1301 were encoded by regions 2, 3, and 4 (Fig. 2).

We conducted a thorough survey of the NY1301 genome to identify genes related to transferable antibiotic resistance and virulence factors following a previously established methodology [16, 17]. Similar to LcY, which has been approved by the FDA as "generally recognized as safe" (GRAS) [23], we did not find any such genes in the NY1301 genome. Specifically, no genes homologous to virulence factors commonly associated with clinically relevant pathogens and no resistance genes or mutations conferring antibiotic resistance were detected. Importantly, none of the resistance genes were linked to mobile elements, such as the NY1301 plasmid.

All participants who completed the open-label trial were included in the safety analysis. Throughout the trial, we observed eight mild adverse events in six subjects, including bruising, eye itching, loose stools, fatigue, cold symptoms, and increased ALT/AST levels. However, the principal investigator deemed these events unrelated to the test treatment. We also observed some slight but statistically significant changes in the parameters during the hematology, blood biochemistry, and somatometry tests (Table 4). Importantly, none of these changes were deemed clinically relevant, indicating that these events were unrelated to the test treatment with large amounts of NY1301.

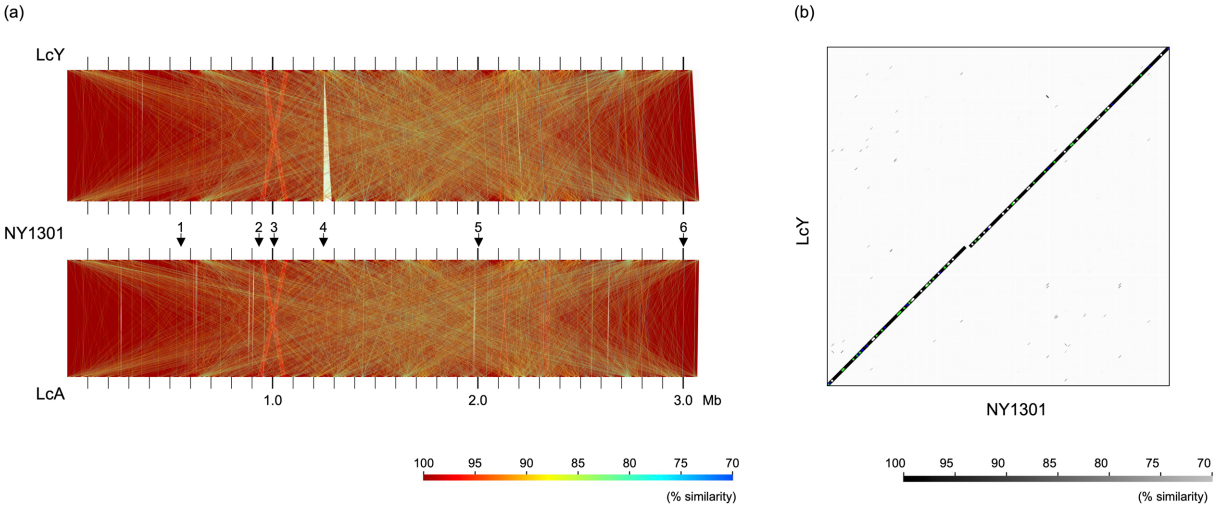

Fig. 1. Genome comparison of closely related *Lacticaseibacillus paracasei* strains. Chromosome comparisons by pairwise blastn (a) and MUMmer (b) are presented, and the similarity is shown by a color code in the figure. Arrows indicate six putative prophage regions in NY1301 in the pairwise blastn comparisons (a). Colored dots indicate the positions of SNPs and InDels in the MUMmer comparisons (b), with different colors representing different types of variations (white for transitions, green for transversions, and blue for insertions and deletions).

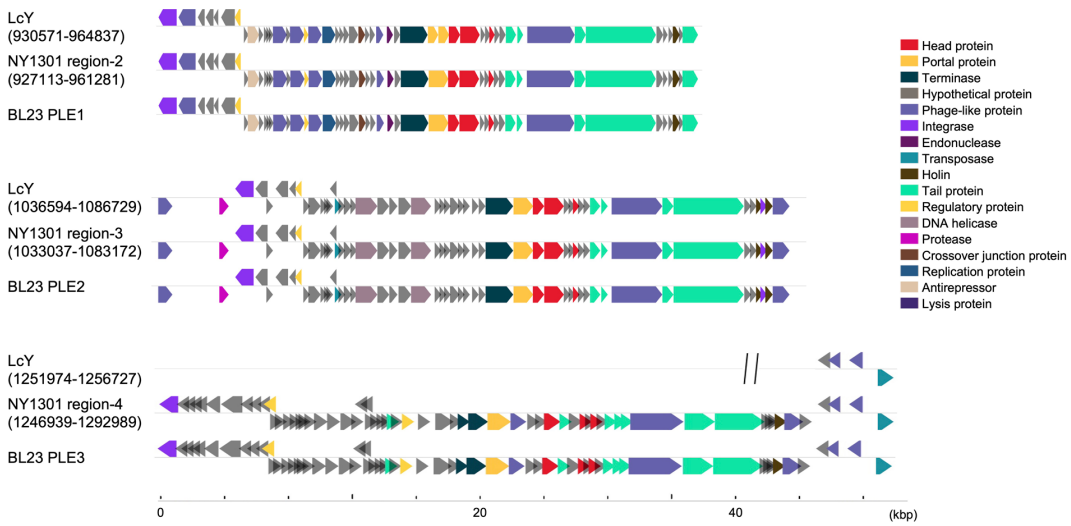

Fig. 2. Genome organization of putative prophages in closely related *Lacticaseibacillus paracasei* strains. Putative prophage regions are indicated by colored arrows, with each arrow representing a predicted gene within the respective prophage region. The colors of the arrows correspond to the predicted functions of each gene.

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Table 4. Effects of excessive intake of NY1301 on hematology and blood chemistry parameters

Item	Pretrial	Week 2	Week 4	Post trial
White blood cell (number/μL)	5,485.0 ± 1,424.3	5,650.0 ± 1,095.7	5,140.0 ± 1,225.3	5,485.0 ± 1,310.0
Red blood cell (number × 10^4 μL)	477.1 ± 38.7	478.0 ± 40.0	472.6 ± 37.0	466.4 ± 39.8*
Hemoglobin (g/dL)	14.32 ± 1.30	14.40 ± 1.37	14.11 ± 1.21*	14.14 ± 1.40
Hematocrit (%)	44.27 ± 3.11	44.59 ± 3.57	44.07 ± 2.86	43.43 ± 3.46*
Platelet (number × 10^4 μL)	27.13 ± 4.55	26.55 ± 5.06	26.76 ± 4.79	26.11 ± 5.12*
AST (U/L)	19.5 ± 4.9	19.1 ± 7.8	20.8 ± 8.5	19.8 ± 6.8
ALT (U/L)	15.2 ± 7.0	16.0 ± 8.9	18.5 ± 19.2	16.1 ± 9.7
LDH (U/L)	166.6 ± 25.6	168.8 ± 29.3	171.3 ± 24.3	167.2 ± 26.0
ALP (U/L)	212.7 ± 45.0	208.5 ± 49.5	203.9 ± 47.8	204.8 ± 47.0
γ-GTP (U/L)	21.1 ± 13.1	22.2 ± 11.5	21.5 ± 13.6	21.6 ± 13.7
Total bilirubin (mg/dL)	0.81 ± 0.38	0.80 ± 0.32	0.89 ± 0.38*	0.85 ± 0.38
Total protein (g/dL)	7.21 ± 0.34	7.12 ± 0.38	7.00 ± 0.34*	7.19 ± 0.31
Albumin (g/dL)	4.44 ± 0.25	4.27 ± 0.30*	4.37 ± 0.26	4.42 ± 0.26
Creatinine (mg/dL)	0.759 ± 0.157	0.729 ± 0.138*	0.733 ± 0.142*	0.743 ± 0.150
Blood urea nitrogen (mg/dL)	12.98 ± 2.60	12.07 ± 2.92*	11.72 ± 2.69*	11.54 ± 2.90*
Uric acid (mg/dL)	5.00 ± 1.23	4.96 ± 1.10	4.92 ± 1.11	4.92 ± 1.20
Glucose (mg/dL)	84.4 ± 8.7	85.4 ± 7.0	83.4 ± 6.1	85.8 ± 7.6
Triglycerides (mg/dL)	97.6 ± 60.3	105.7 ± 63.9	105.5 ± 74.2	101.1 ± 66.0
Total cholesterol (mg/dL)	202.7 ± 23.7	197.1 ± 27.8	199.2 ± 30.7	195.9 ± 30.2
HdL-C (mg/dL)	64.8 ± 13.9	61.3 ± 14.4*	61.1 ± 14.4*	64.0 ± 15.9
LdL-C (mg/dL)	120.8 ± 23.5	116.2 ± 27.0*	119.4 ± 29.9	113.3 ± 28.4*
Na (mEq/L)	142.1 ± 1.6	142.0 ± 2.2	141.0 ± 1.3*	142.8 ± 1.8
K (mEq/L)	4.17 ± 0.27	4.33 ± 0.23*	4.13 ± 0.21	4.24 ± 0.26
Cl (mEq/L)	105.1 ± 1.7	105.2 ± 1.9	104.4 ± 1.8	105.6 ± 1.2

Each value represents the mean ± SD of 20 participants. *p<0.05 using paired Student's t-test compared to the respective pretrial values. AST: aspartate transaminase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; γ-GTP: γ-glutamyltranspeptidase; HdL-C: high-density lipoprotein; LdL-C: low-density lipoprotein cholesterol.

DISCUSSION

The probiotic attributes of NY1301 were comprehensively characterized, and a comparison with the closely related LcA and LcY probiotic strains was conducted using genomic tools. Our findings indicate that the strains are highly similar, differing primarily because of minor chromosomal re-arrangements, substitutions, insertions, and deletions, suggesting a very recent common ancestor, a finding which is consistent with previous observations in LcY and LcA [19]. Despite their genomic similarities, these strains reportedly had distinct historical trajectories, possibly leading to varying evolutionary changes over time. Additionally, the differences between genomes may not be directly reflected at the phenotypic level. Indeed, previous studies have demonstrated considerable metabolic profile variations between LcA and LcY, indicating non-identical phenotypes [20]. Further investigations are warranted to offer a more comprehensive understanding of the mechanisms of action and beneficial properties of NY1301. Future studies employing metabolomic and proteomic approaches will enable deeper characterization of the strain at multiple levels, providing valuable insights into its unique properties and potential health benefits. Such research will contribute to a more holistic view of the functionality of NY1301, facilitating its informed and targeted application in various probiotic-based interventions.

On the NY1301 chromosome, we identified six putative prophages that were designated as regions 1–6 and were similar to known PLE1–PLE6 prophages in BL23. PLE2 in BL23 is spontaneously induced at low levels and does not enter a lytic state during bacterial growth under standard culture conditions [21, 22]. PLE2 also plays a substantial role in the production and release of membrane vesicles with various functions [22]. Furthermore, PLE1 and PLE3 reportedly function as satellite prophages by interacting with PLE2 [22]. Data from the qPCR analysis under standard culture conditions demonstrated the expression of region-3-encoded genes in NY1301. Additionally, purified prophage particles from the culture supernatant were observed in transmission electron microscopy images of negative staining (data not shown), supporting the notion that coordinated prophage induction in NY1301 behaves similarly to that in PLE1, PLE2, and PLE3 in BL23 cells. The presence of these prophages appears to be a natural aspect of NY1301, and their behavior aligns with that observed in BL23. The prophages in NY1301 do not conclusively cause inherent technological problems in routine manufacturing processes.

Throughout the open-label trial, our findings supported the notion that it is safe to consume large amounts of NY1301 (at a concentration of 3.0 × 10^{11} cfu) for four weeks. Overall, the results of the open-label trial, in conjunction with other *in vivo* studies, and the long history of human consumption without any established risk to humans provide robust evidence that reaffirms the safety status of NY1301. The accumulated safety data, along with the absence of antibiotic resistance and virulence factors in the genome, further confirmed the safety profile of NY1301 as a probiotic in humans.

In conclusion, the genetic homogeneity between NY1301 and the closely related strains LcA and LcY, with minor genomic variations, indicates a common ancestry and a potentially

shared functional core. The presence of putative prophages in NY1301, similar to those observed in LcA and LcY, suggests a stable and natural aspect of the genome that is not linked to inherent technological issues in the manufacturing processes. Overall, our findings underscore the potential of NY1301 as a safe and effective probiotic strain with substantial implications in promoting gut health, immunity, and overall well-being. As further research continues to explore its specific mechanisms of action and health-promoting properties, we believe that NY1301 will be a promising probiotic option in the medical and consumer healthcare sectors, facilitating evidence-based probiotic use and personalized interventions.

DATA AVAILABILITY

The genome sequence of NY1301, consisting of one chromosome and one plasmid, was deposited in the DDBJ database under accession numbers AP028677 and AP028678.

CONFLICTS OF INTEREST

Funding for this study was provided by Nissin York Co., Ltd. and Nissin Foods Holdings Co., Ltd. Masanori Fukao and Shuichi Segawa are associated with Nissin York Co., Ltd., while Atsushi Oki is affiliated with Nissin Foods Holdings Co., Ltd.

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REFERENCES

1. Sugahara H, Hirota T. 2021. Chapter 3—Probiotic beverages in Japan (some history and current developments). *In* Probiotic Beverages, Panda SK, Kellershohn J, Russell I (eds), Academic Press, Cambridge, pp. 35–48.
2. Azuma Y, Sato M. 2001. *Lactobacillus casei* NY1301 increases the adhesion of *Lactobacillus gasseri* NY0509 to human intestinal caco-2 cells. Biosci Biotechnol Biochem 65: 2326–2329. [Medline] [CrossRef]
3. Azuma Y, Ito K, Ohki A, Inoue A, Inoue K, Sato M, Benno Y. 2001. Effects of a milk drink fermented with *Lactobacillus gasseri* NY0509 and *Lactobacillus casei* NY1301 on fecal microflora in healthy volunteers. Nippon Shokuhin Kagaku Kogaku Kaishi 48: 35–43. [CrossRef]
4. Nakamura E, Tatsuaki T, Tagawa K, Segawa S, Ueda K, Saito J. 2020. Intake of a *Lactobacillus paracasei* subsp. *paracasei* NY1301-containing beverage improves intestinal flora compositions in constipated subjects—a randomized, placebo-controlled, double blind, parallel-group study. Jpn Pharmacol Ther 48: 1793–1803.
5. Segawa S, Tagawa K, Okabe Y, Nagashima H. 2021. Effect of beverage containing *Lactobacillus paracasei* subsp. *paracasei* NY1301 on fatigue and quality of sleep—a randomized, double–blind, placebo–controlled parallel study. Jpn Pharmacol Ther 49: 2135–2148.
6. Turkmen N, Akal C, Özer B. 2019. Probiotic dairy-based beverages: a review. J Funct Foods 53: 62–75. [CrossRef]
7. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18: 821–829. [Medline] [CrossRef]
8. Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, *et al.* 2014. Efficient *de novo* assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 24: 1384–1395. [Medline] [CrossRef]
9. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. Genome Biol 5: R12. [Medline] [CrossRef]
10. Tanizawa Y, Fujisawa T, Kaminuma E, Nakamura Y, Arita M. 2016. DFAST and DAGA: web-based integrated genome annotation tools and resources. Biosci Microbiota Food Health 35: 173–184. [Medline] [CrossRef]
11. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57: 81–91. [Medline] [CrossRef]
12. Ohtsubo Y, Ikeda-Ohtsubo W, Nagata Y, Tsuda M. 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. BMC Bioinformatics 9: 376. [Medline] [CrossRef]
13. Liu B, Zheng D, Zhou S, Chen L, Yang J. 2022. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res 50 D1: D912–D917. [Medline] [CrossRef]
14. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, *et al.* 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res 51 D1: D690–D699. [Medline] [CrossRef]
15. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, *et al.* 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75: 3491–3500. [Medline] [CrossRef]
16. Fukao M, Oshima K, Morita H, Toh H, Suda W, Kim SW, Suzuki S, Yakabe T, Hattori M, Yajima N. 2013. Genomic analysis by deep sequencing of the probiotic *Lactobacillus brevis* KB290 harboring nine plasmids reveals genomic stability. PLoS One 8: e60521. [Medline] [CrossRef]
17. Clewell A. 2019. Notice to US food and drug administration of the conclusion that the intended use of *Lactobacillus plantarum* ECGC 3110402 (LPLDL®) is generally recognized as safe. GRAS Notice No 000847. ProBiotix Health Plc., Wakefield.
18. Wishart DS, Han S, Saha S, Oler E, Peters H, Grant JR, Stothard P, Gautam V. 2023. PHASTEST: faster than PHASTER, better than PHAST. Nucleic Acids Res 51 W1: W443–W450. [Medline] [CrossRef]
19. Douillard FP, Kant R, Ritari J, Paulin L, Palva A, de Vos WM. 2013. Comparative genome analysis of *Lactobacillus casei* strains isolated from Actimel and Yakult products reveals marked similarities and points to a common origin. Microb Biotechnol 6: 576–587. [Medline] [CrossRef]
20. Douillard FP, Ribbera A, Järvinen HM, Kant R, Pietilä TE, Randazzo C, Paulin L, Laine PK, Caggia C, von Ossowski I, *et al.* 2013. Comparative genomic and functional analysis of *Lactobacillus casei* and *Lactobacillus rhamnosus* strains marketed as probiotics. Appl Environ Microbiol 79: 1923–1933. [Medline] [CrossRef]
21. Dieterle ME, Fina Martin J, Durán R, Nemirovsky SI, Sanchez Rivas C, Bowman C, Russell D, Hatfull GF, Cambillau C, Piuri M. 2016. Characterization of prophages containing "evolved" Dit/Tal modules in the genome of *Lactobacillus casei* BL23. Appl Microbiol Biotechnol 100: 9201–9215. [Medline] [CrossRef]
22. da Silva Barreira D, Lapaquette P, Novion Ducassou J, Couté Y, Guzzo J, Rieu A. 2022. Spontaneous prophage induction contributes to the production of membrane vesicles by the gram-positive bacterium *Lacticaseibacillus casei* BL23. MBio 13: e0237522. [Medline] [CrossRef]
23. Heimbach JT. 2012. Generally recognized as safe (GRAS) determination for the use of *Lactobacillus casei* strain Shirota as a food ingredient. GRAS Notice No 000429. Yakult Honsha Co., Tokyo.

