Original article

Antibacterial activity of the probiotic candidate *Lactobacillus gasseri* against methicillin-resistant *Staphylococcus aureus*

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Abstract

Purpose: The objective of this study was to verify growth suppression of methicillin-resistant *Staphylococcus aureus* (MRSA) involved in ventilator-associated pneumonia (VAP) aggravation by the probiotic candidate bacterium *Lactobacillus gasseri* (*L. gasseri*) in the oral cavity.

Materials and Methods: *L. gasseri* YIT 12321 (*Lg* YIT 12321) and MRSA JCM 8702 (MRSA-JCM) were used as test bacteria. Both *Lg* YIT 12321 and MRSA-JCM were cultured in lactobacilli MRS medium, and MRSA-JCM was also cultured in TS medium anaerobically at 37˚C for 18-24 hours. The antibacterial activity of *Lg* YIT 12321 against MRSA-JCM was examined by competition assay and radial diffusion assay (RDA). A fraction obtained from the *Lg* YIT 12321 culture supernatant by 50% (w/v) saturated ammonium sulfate (50% sat. ammonium) precipitation was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Results: In the competition assay, inhibition of MRSA-JCM proliferation by *Lg* YIT 12321 was observed. Moreover, it was confirmed that the growth of MRSA-JCM was inhibited by neutralized culture supernatant of *Lg* YIT 12321 in the RDA, suggesting that the growth of MRSA-JCM was suppressed by an antibacterial substance produced by *Lg* YIT 12321.

Conclusion: *Lg* YIT 12321 is a candidate probiotic that produces a class II bacteriocin that suppresses the growth of MRSA-JCM.

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Key Words: Bacteriocin, *L. gasseri*, MRSA, probiotics, ventilator-associated pneumonia

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), frequently isolated in hospitals especially intensive care unit (ICU) in more than 100 countries, is responsible for ventilator-associated pneumonia (VAP) [1-3]. The death rate of VAP reportedly increases with advanced age [4]. According to the literature, *Staphylococcus aureus* (*S. aureus*) is more likely to colonize the nasal cavity than the pharynx, and patients receiving intranasal or intravenous nutrition have a 6- to 10-fold higher incidence of MRSA lung infections than patients receiving airway nutrition, and this is believed to be related to the higher death rate [5]. Once *S. aureus* forms a biofilm, the efficacy of antibiotics such as linezolid decreases [6,7]. VAP remains an important cause of morbidity and mortality in mechanically ventilated patients, even though the incidence of VAP has decreased over the past several years in the United States. It is estimated that VAP is responsible for approximately ~27-47% of ICU acquired infections [8]. A prospective cohort study conducted at the central ICU of the Kragujevac Clinical Center in Serbia identified 620 patients with VAP (61.8%), which would also result in little reduction in morbidity [9]. VAP is an important clinical problem in patients using artificial respirators during the perioperative period, with bacterial pneumonia potentially developing within 48 hours of the start of artificial respiration under tracheal intubation.

Currently, perioperative management approaches emphasize oral care, hand disinfection, and prevention, and perioperative management such as antibiotic administration, but the risk could be reduced further by identifying the cause and enhancing the natural microbial flora through the application of probiotics [10,11]. At least seven newly isolated lactic acid bacterial strains have shown promising properties for use as probiotics, either alone or as a part of a probiotic formula, to improve oral health [12]. In addition, the incidence of VAP was reduced significantly following the administration of various probiotic lactobacilli, and the antibacterial activity of *Lactobacillus gasseri* (*L. gasseri*) against intranasal MRSA in hospitalized patients was demonstrated in vitro [13,14]. It is thought that use of the candidate probiotic bacterium *L. gasseri* to suppress the growth of VAP-causing MRSA is a viable alternative to the use of traditional antibacterial agents. In the oral health field, many trials have reported reductions in the incidence of oral diseases following the administration of probiotics [15]. Several probiotic candidates were isolated from the oral cavity of healthy subjects [16], and the characteristics of three probiotic candidates were reported [10,11,17]. This study aimed to verify suppression of the growth of VAP-associated MRSA by the probiotic candidate bacterium *L. gasseri* in the oral cavity.

Materials and Methods

Bacterial strains and growth conditions

Lactobacillus gasseri YIT 12321 (*Lg* YIT 12321), *Lactobacillus crispatus* YIT 12319 (*Lc* YIT 12319), *Lactobacillus crispatus* YIT12945 (*Lc* YIT12945), and *Lactobacillus fermentum* YIT 12320 (*Lf* YIT 12320) were obtained from the stock culture collection of the Microbiological Research Department, Yakult Central Institute (Kunitachi, Japan) and Tsurumi University (Yokohama, Japan). MRSA-JCM was provided by the RIKEN BRC (Tsukuba, Japan) through the National Bio-Resource Project of the MEXT, Japan. All *Lactobacillus* species were cultured in Difco lactobacilli MRS Broth (MRS broth; Becton, Dickinson and Co., Sparks, MD, USA), and *Lg* YIT 12321 was also cultured using modified MRS (mod-MRS). Mod-MRS was prepared by mixing all components used in MRS except for Tween 80. Oleic acid was substituted for Tween 80. MRSA-JCM was cultured in Bacto Tryptic Soy Broth (TS broth; Becton, Dickinson and Co.). All bacteria were incubated in an anaerobox (Concept Mini, Ruskinn, Central Scientific Commerce, Inc., Tokyo, Japan) under an atmosphere comprised of 80% N_2, 10% CO_2, and 10% H_2 at 37˚C for 18-24 hours.

Confirmation of antibacterial activity

Antibacterial activity of *Lg* YIT 12321 against MRSA-JCM was assessed by competition assay and RDA. Competition assays were performed to observe the growth-suppression effects of four candidate oral probiotic bacteria (*Lg* YIT 12321, *Lc* YIT 12319, *Lc* YIT12945, *Lf* YIT 12320) against MRSA-JCM using agar plates containing a mixture of MRS broth and TS broth media at a ratio of 1:1. Each pair of competitive species was inoculated onto the plate at the same time in close proximity (6 mm apart) using 5 μL of an overnight culture after adjusting the concentration to an $OD_{540nm} = 0.5$. The plates were incubated at 37˚C anaerobically for 18 hours before bacterial growth was inspected and scored.

A series of RDAs were performed to investigate the antimicrobial activities of the four candidate probiotic oral bacteria (*Lg* YIT 12321, *Lc* YIT 12319, *Lc* YIT12945, *Lf* YIT 12320) against MRSA-JCM using two layers of agarose gel. For the underlay gel, an agarose solution was prepared containing 0.06 g of TS broth, 1.5 g of agarose, and 100 mL of distilled water (DW) in a conical flask, which was then boiled in a water bath for 5 minutes. The agarose solution was then transferred to 15 mL culture tubes (10 mL each aliquot) and autoclaved. Next, the tubes were placed into a water bath set at 50˚C to allow the agarose solution to cool. MRSA-JCM was grown in 4 mL of TS broth overnight (as described above), and then 100 μL of the MRSA-JCM culture was added to a 10 mL aliquot of agarose solution. After dispersing the bacteria by gentle mixing, the mixture was poured into a 10 cm diameter sterile Petri dish and allowed to solidify in a clean bench. A series of wells (2 mm in diameter) was punched in the agar, and 5 μL of each test solution was pipetted into a designated well. The plates were kept in the clean bench for 10 minutes at room temperature to allow the experimental solutions to diffuse. For the overlay gel that provides nutrients for microbial growth, an agarose solution was prepared as described above, except that it only contained TS broth (6 g in 10 mL of DW, and 1.5 g of agar). This solution was then poured over the base and allowed to solidify in a clean bench. The plates were then turned over and incubated under anaerobic conditions in an atmosphere of 80% N_2, 10% CO_2, and 10% H_2 at 37˚C for 18 hours, at which time the diameters of the clear bacterial growth inhibition zones around the wells were measured using a slide caliper. The salting out samples were obtained from *Lg* YIT 12321 culture supernatant by stepwise precipitation with 25%, 50%, and 80% (w/v) saturated ammonium (25%, 50%, 80% sat. ammonium). The samples used to assess the antimicrobial activity of the culture supernatant of oral probiotic bacteria against MRSA-JCM observed by RDA included *Lg* YIT 12321 supernatant of MRS, *Lg* YIT 12321 supernatant of mod-MRS, *Lg* YIT 12321 mod-MRS (25% sat. ammonium), *Lg* YIT 12321 *m*od-MRS (50% sat. ammonium), *Lg* YIT 12321 mod-MRS (80% sat. ammonium), *Lg* YIT 12321 mod-MRS (25% ammonium chloroform), *Lg* YIT 12321 mod-MRS (50% ammonium chloroform), and *Lg* YIT 12321 mod-MRS (80% ammonium chloroform). The *Lg* YIT 12321 mod-MRS (25, 50, and 80% sat. ammonium) samples had been subjected to dialysis against phosphate-buffered saline (PBS). After mixing *Lg* YIT 12321 mod-MRS (25, 50, 80% sat. ammonium) with chloroform at a 1:1 ratio, the mixture was centrifuged (1,710 × *g*) at 4˚C for 5 minutes, and the chloroform lower layer was collected. The chloroform layer was then subjected to centrifugal evaporation after the addition of PBS, and the concentrated sample obtained after filter sterilization served as *Lg* YIT 12321 mod-MRS (50% ammonium chloroform).

Purification of bacteriocin-like substance

One liter of the supernatant of *Lg* YIT 12321 culture was precipitated with 25-80% sat. ammonium with stirring. After precipitation with 25% sat. ammonium, 50% sat. ammonium was added to the resulting supernatant, and the precipitate was collected. Next, 80% sat. ammonium was added to the supernatant of the 50% sat. ammonium fraction. Precipitated proteins were collected by centrifugation (3,970 × *g*) at 4˚C for 20 minutes and then resuspended in 14 mL of PBS (pH 7.4). The precipitated proteins were dialyzed twice against 1 L of PBS for 24 hours using a dialysis bag. The dialyzed proteins were mixed vigorously with an equal volume of chloroform and then centrifuged (1,710 × *g*) at 4˚C for 15 minutes. The remaining precipitated proteins were also extracted with an equal volume of chloroform. The total organic

phase was evaporated using a rotary evaporator.

Antibacterial effect analyzed in co-culture

The antibacterial effect was evaluated under mono- and co-culture conditions at 0, 2, 4, 8, and 16 hours (n = 5 for both mono- and co-culture). Cells were cultured as follows for each replicate. The optical density at 540 nm of MRSA-JCM and *Lg* YIT 12321 pre-culture were adjusted to 0.5. Each bacterium was cultured in the specially prepared culture medium (MRS:TS = 1:1) under anaerobic condition. In the case of co-culture, same volume of both bacterial suspensions was mixed and cultured in the medium (MRS:TS = 1:1). At each time point, 1 mL aliquot of culture was centrifuged (1,710 × *g*) at 4˚C for 15 minutes, and the bacterial pellet was suspended in 1 mL of chilled PBS to be analyzed for viability, as described previously [11]. Each bacterial cell suspension (1 mL) was diluted to 10^{-3}~10^{-5} before plating. Treated and control samples were plated on petri dishes containing MRS or TS agar medium using a spiral plating instrument (Eddy Jet2, IUL, Barcelona, Spain). After 48 hours of incubation under anaerobic condition at 37˚C, the Colony Forming Unit (CFU) was determined.

Determination of protein concentration, tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein content was estimated using a Quick Start Protein Assay kit (Bio-Rad Laboratories Inc., Tokyo, Japan), according to the manufacturer's instructions. Tricine SDS-PAGE was performed on a 15% acrylamide gel (e-PAGEL, ATTO Corp., Tokyo, Japan). Ten microliters of each *Lg* YIT 12321 sample was applied to the gel and electrophoresed. The gel was cut into two parts; one was stained with Ezstain (AE-1340 Ezstain Aqua, ATTO Corp.), and the other was rinsed in 0.1%(v/v) Triton X-100 in sterile water for 30 minutes. One gel section was then overlaid on a test agar plate containing MRSA-JCM (500 μL) indicator and incubated under anaerobic conditions at 37˚C for 18 hours. At the same time, another part of the gel, corresponding to the molecular weight of 6.5 kDa, was excised and the protein was analyzed by nano Liquid Chromatography-Mass spectrometry (nano LC-MS/MS), with protein identification performed using Mascot Search (Fujifilm Wako Pure Chemical Industries, Ltd., Osaka, Japan). The amount of protein was calculated as mentioned above by measuring the absorbance at OD_{655nm} using a spectrophotometer (Bio-Rad, iMark). Bovine serum albumin (Fujifilm Wako Pure Chemical Industries, Ltd., grade 1) was used as a standard control.

Characterization of the bacteriocin-like substance

The heat stability and proteinase sensitivity of the bacteriocin-like substance were also investigated. Partially purified protein was incubated with proteinase K (1 mg mL^{-1}) or trypsin (1 mg mL^{-1}) in PBS at 37˚C for 3 hours. Remaining activity was assessed by measuring the diameter of inhibition zones using a RDA, and the percentage of activity remaining was calculated based on a standard curve semi-logarithmic plot of diameter-dose responses.

Results

Inhibition of MRSA-JCM growth as determined by competition assay and RDA

The results of competition assay are shown in Fig. 1. Only *Lg* YIT 12321 suppressed the growth of MRSA-JCM with consistent scores, with all test showing (++) results, and *Lg* YIT 12321 demonstrated the strongest effect against MRSA-JCM.

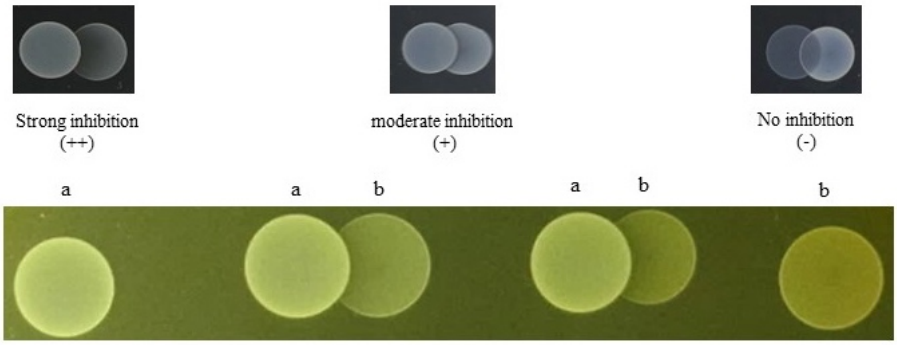

Fig. 1 Growth inhibition scoring and the results of competition assays (n = 3 for each experiment); (a) *Lg* YIT 12321, (b) MRSA-JCM

Compared with other strains examined, *Lg* YIT 12321 produced the largest inhibition of MRSA-JCM growth, as shown in Fig. 2. The other three strains (*Lc* YIT 12319, *Lc* YIT12945, and *Lf* YIT 12320) did not produce detectable clear zones

in the RDA. Strong antimicrobial activity was observed in the 50% sat. ammonium precipitate and chloroform-concentrated sample of 50% sat. ammonium. The RDA results are summarized in Table 1. Samples (1), (3), (4), and (7) indicated in Table 1 exhibited inhibition of MRSA-JCM growth. In contrast, no antibacterial activity was detected in the 25, 50, and 80% *Lg* YIT 12321 chloroform-water layers. No activity was observed with proteinase- and trypsin-treated samples or proteinase and trypsin alone. The active substance produced by *Lg* YIT 12321 was found to be heat resistant (Table 2).

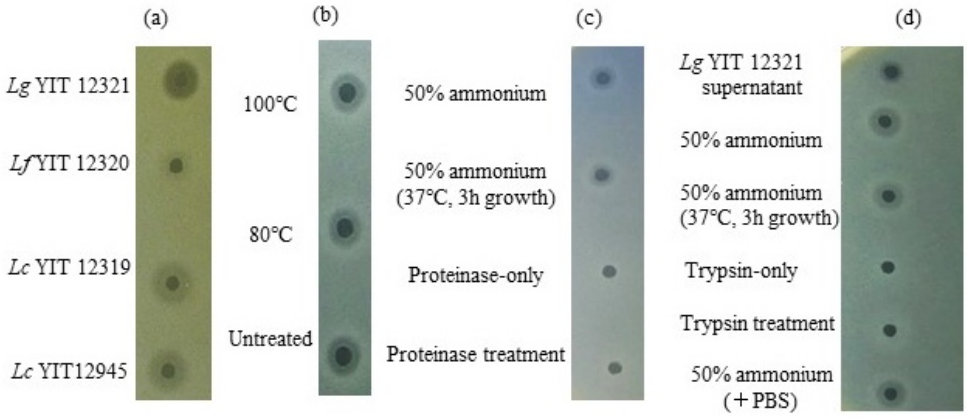

Fig. 2 Antibacterial activity of *Lactobacillus* culture supernatant samples against MRSA. Samples of *Lg* YIT 12321 supernatant were treated under various conditions; (a) Antibacterial activity of *Lactobacillus* isolates, (b) Activity of *Lg* YIT 12321 after heat treatment, (c) Activity of *Lg* YIT 12321 after proteinase K treatment, and (d) Activity of *Lg* YIT 12321 after trypsin treatment (n = 3 for each experiment). 50% saturated ammonium sulfate (50% sat. ammonium)

Table 1 Antibacterial activity of culture supernatants of *Lg* YIT 12321 against MRSA, as determined by RDA

Samples	Antibacterial activity against MRSA-JCM*
(1) MRS culture supernatant	1.08 ± 0.12
(2) mod-MRS culture supernatant	n.d.
(3) 25% saturated ammonium sulfate fraction	0.87 ± 0.21
(4) 50% saturated ammonium sulfate fraction	1.44 ± 0.19
(5) 80% saturated ammonium sulfate fraction	n.d.
(6) 25% saturated ammonium sulfate and chloroform fraction	n.d.
(7) 50% saturated ammonium sulfate and chloroform fraction	2.39 ± 0.08
(8) 80% saturated ammonium sulfate and chloroform fraction	n.d.

Samples (3)-(8) were obtained from mod-MRS culture supernatant.
*diameter of clear-zone expressed in mm; n = 6 for each experiment; n.d., not detected

Table 2 Effect of various treatments on antibacterial activity of 50% sat. ammonium precipitate

Radial	Activity
Control	1.87 ± 0.12
100˚C treatment	1.86 ± 0.11
80˚C treatment	1.89 ± 0.15
Protease treatment	0
Trypsin treatment	0

All samples are 50% saturated ammonium sulfate fraction. n = 3 for each experiment

Antibacterial effect detected with CFU in co-culture

In *Lg* YIT 12321 and MRSA-JCM mono-cultures, both *Lg* YIT 12321 and MRSA-JCM increased with time (Fig. 3). In co-culture of *Lg* YIT 12321 and MRSA-JCM, MRSA-JCM colonies decreased after 8 hours and MRSA-JCM growth was inhibited after 16 hours (Fig. 4).

Characterization of the bacteriocin-like substance of *Lg* YIT 12321

Lg YIT 12321 exhibited antimicrobial activity against MRSA-JCM. A few visible bacterial cells were observed by microscope in a clear zone in the RDA. The quantitative results were reflected in the RDA carried out at the same time as protein quantification, and the activity of the 25% sat. ammonium fraction was 2.33 times higher than that of the *Lg* YIT 12321 supernatant fraction, whereas the activity of the 50% sat. ammonium fraction was 2.84 times higher. The 25, 50, and 80% sat. ammonium fractions contained 0.85, 0.35, and 0.0005 (mg protein mL^{-1}), respectively. The molecular

weight of the active component was 6.5 kDa based on tricine SDS-PAGE (15%) with a low-range molecular weight standard (Fig. 5).

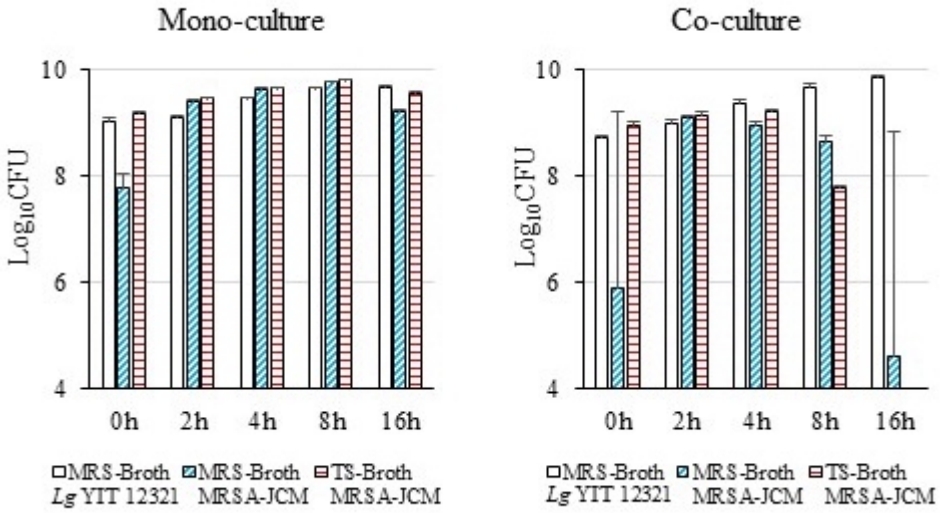

Fig. 3 (left) Growth of MRSA-JCM and *Lg* YIT 12321 in mono-culture determined by CFU (n = 5 for each experiment)
Fig. 4 (right) Effect of co-culture with *Lg* YIT 12321 on the growth of MRSA-JCM determined by CFU (n = 5 for each experiment)

For protein identification, the peptide excised from gel was fragmented with trypsin and analyzed by LC/MS, which revealed a molecular weight of 6,673 Da for a peptide of 65 amino acid residues. The sequence was then compared with those of *L. gasseri* deposited in databases such as National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI-BLAST) and UniProt Knowledgebase (UniProtKB). A total of 58% of the sequence matched proteins in the databases (Fig. 6), with matches to AHI41138, ALX37948, BAM09406, KDA99086, and PKZ91009 in GenBank [18-22]. Of the tryptic fragments of the *Lg* YIT 12321 peptide, three matched peptide sequences stored in the NCBI prot database (Table 3). A search of UniProtKB returned the same result as Mascot Search. The antibacterial *Lg* YIT 12321 peptide was thus identified as bacteriocin, encoded by the *gatX* gene. The primary (citable) accession number is Q9XDR6, whereas the taxonomic identifier is 1596 (NCBI), and the Open Reading Frame (ORF) is C3745-03215. Although encoded by *gatX*, we could not conclude from our analyses whether the peptide is a member of the *gatX* family. The ion score was $-10 \times \log(P)$, where P represents the probability that the observed match is a random event. An individual ion score >55 would indicate identity or extensive homology ($P < 0.05$). The protein score was 93, which was more significant at $P < 0.05$ than the threshold score of 55 (Table 4).

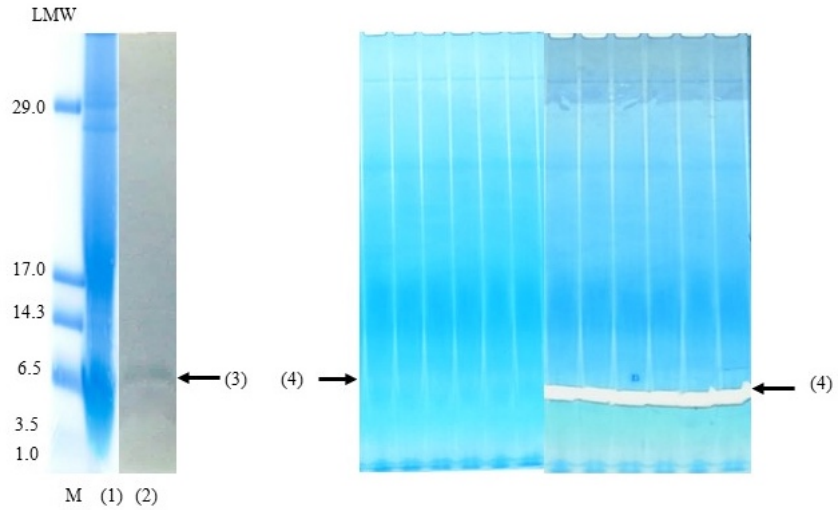

Fig. 5 Tricine SDS-PAGE analysis of partially purified bacteriocin from *Lg* YIT 12321. Lanes: M, molecular mass standard (ATTO AE-1440 EzStandard), (1) 50% sat. ammonium with Ezstain, (2) gel overlaid on 1% agar plate containing MRSA-JCM (500 µL). Arrows: (3) MRSA-JCM growth inhibition band after antibacterial activity staining, (4) Gel band before (left) and after (right) removal for protein identification (samples from 12 lanes were used for the analysis).

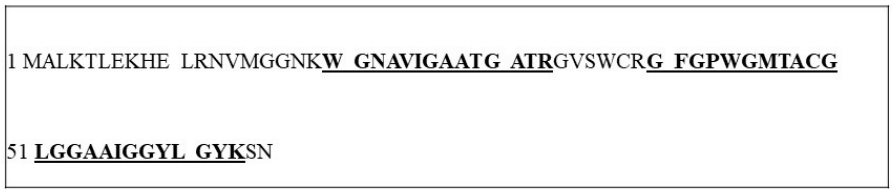

Fig. 6 Complete sequence of *Lg* YIT 12321 bacteriocin (65 amino acid residues). Bold letters are peptide fragment sites matched to the reported sequence for *L. gasseri*.

Table 3 Protein sequences matched to the peptide fragment of *Lg* YIT 12321

Peptide 1 (Query No. 346)	K. WGNAVIGAATGATR.G
Peptide 2 (Query No. 347)	K. WGNAVIGAATGATR.G
Peptide 3 (Query No. 550)	R. GFGPWGMTACGLGGAAIGGYLGYK.S

Table 4 Protein and gene information for *Lg* YIT 12321 obtained from Mascot search and UniProt KB

Sample No.	Protein	gene	MW	Score	Peptide	Coverage	Accession
1	Bacteriocin	gatX gaeX	6,673	93	3	58	Q9XDR6

Discussion

In this study, we demonstrated that *Lg* YIT 12321 exhibits antibacterial activity against MRSA-JCM. The results of competition assays and RDAs demonstrated that *Lg* YIT 12321 inhibits the growth of MRSA-JCM. Sogabe-Ashigaki et al. reported that *Lg* YIT 12321 inhibited the growth of *Haemophilus influenzae* ATCC 9759 in competition assays and RDAs [11]. Nakano et al. also reported that *Lg* YIT 12321 exhibited antibacterial activity against *Streptococcus pneumoniae* ATCC 33400 in competition assays and RDAs [17]. These results suggest that *Lg* YIT 12321 is a probiotic candidate that could be used to inhibit the growth of opportunistic bacterial pathogens in the oral cavity.

The growth of MRSA-JCM was suppressed by neutralized culture supernatant of *Lg* YIT 12321 in a RDA, suggesting that the growth of MRSA-JCM was inhibited by an antibacterial substance produced by *Lg* YIT 12321. The partially purified antibacterial substance of *Lg* YIT 12321 was obtained from the culture supernatant by 50% sat. ammonium precipitation and subsequent chloroform extraction. The molecular mass of the antibacterial substance of *Lg* YIT 12321 was estimated as 6.5 kDa based on tricine SDS-PAGE with low-molecular-weight standards. This antibacterial substance was heat stable but susceptible to proteinases such as trypsin and proteinase K.

The antibacterial substance of *Lg* YIT 12321 isolated in this study was composed of 65 amino acid residues with a molecular weight of 6,673 Da. It was identified as a class II bacteriocin, which are heat-stable polypeptides of 5-10 kDa. The molecular weight of gassericin A from *L. gasseri* LA39 and gassericin B from *L. gasseri* JCM 2124 were reported as 5,652 Da and 4,400 Da, respectively [23,24]. Gassericin T from *L. gasseri* SBT2055 was heat stable and included two peptide chains, GatA (57 amino acid residues) and GatX (48 amino acid residues), with molecular weights of 5,539 Da and 4,761 Da, respectively [18]. The molecular weight of the GatX precursor of gassericin T from *L. gasseri* LA158 was found to be 6,673 Da [25]. Gassericin KT7 from *L. gasseri* KT7, with a molecular weight of 4,500-5,000 Da, was found to be thermostable and sensitive to proteolytic enzymes [26]. Based on a comparison of the characteristics of the *Lg* YIT 12321 antibacterial peptide and reported gassericins, the antibacterial substance identified in this study appears to be a class II bacteriocin. Of the 65 amino acid residues, sequences covering 38 residues completely matched the complete sequence of the GatX component of gassericin T. The 17 N-terminal amino acids appear to be a leader peptide. These results thus indicate that the bacteriocin we identified is similar to the GatX component of gassericin T of the lactacin F family. As indicated above, gassericin T is composed of two peptides, GatX and GatA. However, we did not identify a component similar to GatA in this study. The GatA component itself was thought to exhibit weak or no antibacterial activity. Because our bacteriocin was excised from a gel after SDS-PAGE based on determination of antibacterial activity by staining, it is possible that another component was missed. Additional research will be needed to determine whether the bacteriocin isolated here has a GatA component.

Lg YIT 12321 was originally isolated from the human oral cavity and identified as a probiotic candidate based on its inhibition of the growth of periodontopathic bacteria without increasing the risk of adverse events such as infectious endocarditis [16]. However, whether this organism could be used as a probiotic against multidrug-resistant infectious

bacteria remained unclear until the present study. In this study, *Lg* YIT 12321 was demonstrated to inhibit the growth of MRSA-JCM, an important VAP-causing bacteria associated with perioperative opportunistic infections. Probiotic bacteria such as lactobacilli exhibit relatively good gastrointestinal resistance [27,28], desirable enterocyte adhesion [28], strengthening of the gastrointestinal barrier function [29], and enhancement of the enteric bacterial flora [27-29]. It is well-established that the incidence of VAP can be reduced by altering the composition of the oral flora to inhibit the growth of pathogenic bacteria [29]. Based on these considerations, the use of probiotics would be effective for the prevention of VAP. It would also be effective to take probiotics together with antibiotics such as vancomycin, a first-line agent for treating MRSA, or daptomycin (which is unfortunately inactivated when bound to lung surfactant), in the clinical treatment of VAP or for prophylactic administration and perioperative care in the ICU. Probiotics could contribute to VAP remission.

The bacteriocins reported to date, including that isolated from *L. gasseri*, exhibit low-pH tolerance and resistance to bile salts with respect to their antibacterial activity [30-33]. It was reported that the combination of gassericin A or gassericin T with glycine suppresses the growth of food-spoilage bacteria such as gram-positive organisms isolated from custard cream [34,35]. Some bacteriocins, such as nisin produced by *Lactococcus lactis*, have been added to various foods for the purpose of prolonging the shelf life [36], treating pathogen-derived diseases and cancer [37], or for general health maintenance, including anti-inflammatory potential [38]. Because of the growing application of probiotics in oral care [39], the candidate probiotic *L. gasseri* or its gassericin could be employed as an additional tool in the antibiotic treatment of multidrug-resistant pathogens in the oral cavity. The utility of *Lg* YIT 12321 as an oral probiotic should be confirmed through further research.

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Conflicts of Interest

N. Hanada and S. Imai received research funds from Yakult Central Institute (Kunitachi, Japan). All remaining authors declare no potential conflicts of interest with respect to authorship and publication of this article.

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