Annals of Tropical Research 47 (2):124-135(2025)
https://doi.org/10.32945/atr4729.2025

Original Article

Purification and characterization of a two-peptide bacteriocin with synergistic activity from *Enterococcus faecalis* MGL-3

Rodney H. Perez[1*]

ABSTRACT

Bacteriocins are food-grade bioactive peptides produced by some bacterial strains, including lactic acid bacteria (LAB). Bacteriocins derived from food-grade LAB possess enormous potential for various applications in the food and medical industries. A newly isolated lactic acid bacterium from a fermented vegetable, strain MGL-3, was found to produce two bioactive peptides. In this study, the identification of strain MGL-3 as well as the purification and partial characterization of its bacteriocinogenic peptides are described. Morphological, biochemical, and bioinformatic analyses revealed that strain MGL-3 is an *Enterococcus faecalis* strain. Two bioactive peptides were purified from the cell-free supernatant of strain MGL-3 after a 3-step purification scheme involving salt precipitation and chromatographic techniques. Characterization of these peptides showed that they exhibit relatively weak bioactivity when tested alone but manifest very potent bioactivity when combined, suggesting synergistic activity. The antibacterial spectrum of these peptides was found to target many bacterial strains that are known contaminants in the vegetable pickle industry, highlighting their potential utility in the industry. Nonetheless, further study is needed to confirm the novelty of these peptides, such as the determination of their amino acid sequence and/or the identification of their biosynthetic gene cluster.

Keywords: lactic acid bacteria, bacteriocins, two-peptide bacteriocin, biopreservative

Received: 28 July 2025
Revised: 17 September 2025
Accepted: 30 August 2025
Published: 12 December 2025

[1]National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), College, Laguna 4031, Philippines

*Corresponding Author. Address: National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), College, Laguna 4031, Philippines; Email: rhperez@up.edu.ph

INTRODUCTION

Lactic acid bacteria (LAB) play an indispensable role in many food fermentations. Numerous LAB strains have been shown to positively influence the shelf-life and the nutritional and organoleptic quality of fermented foods (Holzapfel et al., 1995). One of the many attractive attributes of LAB is their ability to produce antimicrobial compounds such as bacteriocins. Bacteriocins are potent antimicrobial peptides that target many industrially relevant strains, including food spoilage and food-borne pathogenic strains (Chen & Hoover, 2003; Cleveland et al., 2001). Bacteriocins from LAB are considered food-grade antimicrobials because of the Generally Regarded as Safe (GRAS) distinction of these microorganisms and their by-products by the Food and Drug Administration of the United States (USFDA, 1988). Aside from its food-grade nature, bacteriocins also possess several desirable properties that enhance their utility as a natural food preservative. These compounds possess inherent tolerance to high thermal conditions and stable bioactivity over a wide pH range common in many food systems. Moreover, the absence of any distinct organoleptic properties that can interfere with the overall food sensory quality further enhances their utility as food preservatives (Perez et al., 2014).

These diverse compounds are typically grouped into two main classes. Class I bacteriocins or lantibiotics (lanthionine-containing antibiotics) are small peptides (<5 kDa) that possess unusual post-translationally modified residues such as lanthionine or 3-methyllanthionine. Class II bacteriocins, the most naturally occurring bacteriocins, are small (<10 kDa), heat-stable peptides that undergo less extensive post-translational modification(s). This group is further subdivided into four subclasses: (1) the "pediocin-like" bacteriocins or class IIa, which are known for their potent anti-listerial bioactivity; (2) the class IIb or the two-component bacteriocins are distinctly known for their synergistic activity, wherein their optimal antimicrobial activity requires the presence of the two peptide components; (3) the class IIc or the circular bacteriocins are known for their high stability and broad antimicrobial spectra; and (4) the heterogeneous group comprising the unmodified, linear, non-pediocin-like bacteriocins makes up the class IId bacteriocins (Cotter et al., 2005; Klaenhammer, 1993).

A new lactic acid bacterial isolate from a fermented vegetable–strain MGL-3–was found to exhibit strong inhibitory activity against different indicator strains after the initial colony overlay assay. Initial tests, such as the heat and enzyme treatment of the cell-free culture supernatant of strain MGL-3, were suggestive of the proteinaceous nature of the antimicrobial substance. In this present study, the identification of strain MGL-3 as well as the establishment of the purification system of the bioactive peptides from its culture supernatant are described. The molecular mass of the bacteriocinogenic peptides is also determined. Although after cross-checking with the bacteriocin-dedicated database, no known bacteriocin has been reported to have the same molecular mass, the amino acid and DNA sequences encoding these bacteriocinogenic peptides still need to be determined to confirm their novelty.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

All LAB strains, including strain MGL-3, were cultivated in de Man Rogosa Sharpe (MRS) medium (HiMedia Ltd., India) and incubated at 30°C for 24h. Non-LAB strains used as indicator strains during bioassays were cultivated in Tryptic Soy Broth (HiMedia), supplemented with 0.6% Yeast Extract (Titan Media Ltd., India) (TSBYE), and incubated at 37°C for 24h. All bacterial cultures were stored in their respective media containing 30% glycerol and kept at −80°C. The strains were reactivated by cultivating them twice prior to every use.

Identification of Strain MGL-3

The initial identification of strain MGL-3 was done using BioMérieux® Vitek2® Identification System (BioMérieux, Marcy l'Etoile, France). The Vitek2® GN cards were set up according to instructions given by BioMérieux®. All reagents and equipment were supplied by the manufacturer. The isolate was introduced to the computer before processing, and the inoculated cards were processed in the instrument within 30min after inoculation. Confirmation of the identity of strain MGL-3 was done through 16S rRNA gene sequencing and through homology search from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) program. Multiple sequence alignment with DNA sequences of known reference bacteria was carried out through the ClustalW algorithm, and the corresponding phylogenetic tree was constructed following the neighbor-joining algorithm using Mega 12 software (Kumar et al., 2024).

Genomic DNA Isolation and PCR Amplification

The genomic DNA of strain MGL-3 was extracted using the GF-1 Bacterial DNA Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's protocol. The genomic DNA concentration was measured using NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA) and standardized to 10ng μL^{-1} using sterile ultrapure water before it was stored at -20°C.

PCR amplification was performed in a 20µL reaction mixture using EmeraldAmp MAX HS PCR Master Mix (TaKaRa Biotechnology, Japan), added with 0.5ng of DNA, and 0.2µM of each 16S rRNA amplification universal primer 1101F (5'-AAC GAG CGC AAC CC-3') and 1407R (5'-GAC GGG CGG TGT GTA C-3'). The PCR reaction was carried out using MultiGene Gradient Thermal Cycler (Labnet International, USA) under the following conditions: an initial denaturation step of 98°C for 2min; 30 cycles consisting of denaturation of 98°C for 10s, 52°C annealing temperature for 30s, and elongation of 72°C for 2min; followed by a final extension step at 72°C for 2min. The amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels in 0.5× TAE buffer with ViSafe Red Gel Stain (Vivantis Technologies, Malaysia). The purified PCR amplicon was then submitted for DNA sequencing.

Bacteriocin Purification

The bioactive peptides were purified from the 200mL culture of strain MGL-3 grown until late exponential-early stationary phase in MRS broth incubated at 30°C. The cells were removed by centrifugation at 6,000rpm for 15min at 4°C. Ammonium sulfate powder was incrementally added until 80% saturation level was reached. Initial tests have shown that at this level of saturation, all bioactive components in the cell-free culture supernatant have been precipitated. The pelleted protein precipitate was then collected after centrifugation at 6,000rpm for 30min. The pellet was then resuspended in 50mM sodium phosphate buffer (pH 5.6) and allowed to pass through the activated OASIS HLB column at a flow rate of 5mL/min. The column was washed with 40% aqueous methanol to remove medium-borne impurities. The active fraction was eluted with 70% aqueous methanol acidified with 0.1% Trifluoroacetic acid (TFA). The eluted fraction was then evaporated to remove the alcohol. Final purification was done through a Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) using a 3 ml RESOURCE RPC column (Amersham Biosciences, Uppsala, Sweden) incorporated in the LC-2000 Plus HPLC system (Jasco, Tokyo, Japan). The active fractions were eluted with a gradient of MilliQ water- acetonitrile containing 0.1% TFA at a flow rate of 1mL min^{-1}. Individual fraction of interest was further purified by reapplying onto the same RP- HPLC column. Purified active fractions were placed in a Speed-Vac Concentrator (Savants, Farmingdale, NY, USA) to thoroughly remove the organic solvent.

Estimation of Bacteriocin Activity

Estimation of the bacteriocin activity of culture supernatant and active fractions during every purification step was determined using the spot-on-lawn method as previously described (Perez et al., 2020). Briefly, 10L of two-fold dilutions of culture supernatant were spotted onto a double-layered agar plate comprising 5mL of TSBYE agar inoculated with an overnight culture of an indicator strain as an upper layer and 10mL of MRS medium supplemented with 1.2% agar (Titan Media) as a bottom layer. *Latilactobacillus sakei* subsp. *sakei* JCM 1157^T was used as an indicator strain unless otherwise mentioned. The titer of antimicrobial activity in the culture supernatant in production experiments was expressed as activity units (AU) per milliliter, in which the reciprocal of the highest dilution at which growth inhibition was still detectable.

Determination of Minimum Inhibitory Concentration (MIC) and Synergistic Activity

The minimum inhibitory concentration (MIC) of the bacteriocin peptides of strain MGL-3, was determined by the spot-on-lawn method as described above, except that the starting concentration of the purified bacteriocin is known prior to the two-fold serial dilution. The purified peptides were quantified using the BCA Protein Assay Kit™ (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions. The MIC is the lowest concentration of the bacteriocin solution that shows detectable growth inhibition in the agar lawn. The initial concentration of each peptide solution before serial dilution was 60µM. An equimolar concentration (1:1) mixture of α and β peptides was tested as described above to determine if the combination of these peptides is synergistic, additive, or

antagonistic. The fractional inhibitory concentration (FIC) indices of these peptides were also calculated to quantify its combination effect.

To further demonstrate and visualize the synergistic activity of the bacteriocinogenic peptides of strain MGL-3, equimolar concentration of each peptide was spotted next to each other in an agar lawn seeded with the indicator strain. A distinct inhibition zone between the spotted regions indicates the synergistic activity of the peptides.

Molecular Mass Determination of Bacteriocin

Molecular mass of purified bioactive peptides was determined using Electrospray Ionization Time of Flight Mass Spectrometry (ESI-TOF MS) with a JMS-T100LC mass spectrometer (JEOL, Tokyo, Japan). The MS detector was set in positive mode ionization using electrospray, desolvation temperature at 260°C, needle voltage at 2000V, orifice voltage at 75V, and ring lens voltage at 10V. The total ion chromatograms were taken in a mass range from m/z 1000 to 3000. The data acquisition was performed using a JEOL MassCenter program (JEOL). The molecular mass of the peptides was calculated from the most abundant ion species detected from each peptide.

RESULTS AND DISCUSSION

Identification of Strain MGL-3

Morphological characteristics of strain MGL-3 showed Gram-positive cocci of around 0.8-1.0m in diameter, which occur singly, in pairs, or in clusters. The cells of strain MGL-3 were non-motile and non-spore forming. In MRS agar plate, colonies were translucent, round shiny and smooth surface, convex with entire margin, yellowish in color and approximately 2mm in diameter. Physiologically, strain MGL-3 was negative for catalase, microaerophilic and had a homofermentative type of fermentation.

The initial efforts for the identification of strain MGL-3 using the BioMérieux® Vitek2® Identification System failed to assign the strain MGL-3 to any particular genus because its substrate assimilation showed contraindicating typical biopattern relative to the type strain (Table 1). Hence, sequencing the 16S rDNA of strain MGL-3 was done to confirm its identity. BLAST hits showed that strain MGL-3 showed 98.91% homology to various *Enterococcus faecalis* strains at 95% query coverage (query submitted on 10/5/2021). This level of sequence similarity suggests the strong likelihood that the strain belongs to this particular bacterial species. According to Tajima et al. (2000), there are no exact 16S rDNA similarity limits for defining specific bacterial taxa, but in general, sequence similarities greater than 98% are sufficient for bacterial species identification.

To confirm the identity of strain MGL-3 (GenBank accession number: PX279126), phylogenetic analysis based on the neighbor-joining algorithm using 16S rRNA sequences of different known *Enterococcus* species, including the type-strain *E. faecalis* JCM 5803^T and other common LAB strains as reference, was done. The result showed that the taxonomic position of strain MLG-3 is very close *to E. faecalis* (Figure 1). Taken together, the aforementioned morphological, physiological characteristics, and phylogenetic analysis are in agreement that strain MGL-3 is an

E. faecalis strain. Nevertheless, the atypical biochemical characteristics of strain MGL-3 suggest that this strain possesses unique characteristics relative to most members of this bacterial species.

Table 1. Biochemical characteristics of strain MGL-3 based on substrate for assimilation test using Vitek2®

Substrate for Assimilation		Result[a]		Substrate for Assimilation		Result[a]	
		MGL-3	Type strain[b]			MGL-3	Type strain[b]
1	Ala-Phe-Pro-arylamidase	-	-	22	saccharose/sucrose	+	+
2	D-galactose	-	+	23	D-trehalose	+	+
3	ornithine decarboxylase	-	-	24	citrate (sodium)	+	+
4	phenylalanine arylamidase	+	+	25	5-bromo-4-chloro-3-indoxyl-beta-glucuronide	-	-
5	arginine GP	+	+	26	L- lactate alkalinization	-	(-)
6	pyruvate	(-)	+	27	α-glucosidase	+	+
7	β- galactosidase	(+)	+	28	D-sorbitol	+	+
8	L-pyrrolidonyl-arylamidase	+	+	29	α-galactosidase	-	-
9	succinate alkalinasation	-	-	30	glycine arylamidase	-	-
10	tyrosine arylamidase	+	(+)	31	D-malate	-	+
11	D-glucose	+	+	32	D-ribose	+	+
12	β-glucosidase	+	+	33	maltotriose	+	(+)
13	D-maltose	+	+	34	L-glutamine	-	+
14	D-mannitol	+	+	35	phenylphosphonate	-	-
15	β-xylosidase	-	+	36	β-D-fucosidase	-	-
16	O/129 resistance (comp.vibrio)	+	+	37	coumarate	+	+
17	L-proline arylamidase	-	-	38	2-keto-D-gluconate	-	(+)
18	lipase	+	-	39	esculin hydrolyse	+	+
19	α-mannosidase	-	+	40	ellman	+	+
20	D-melezitose	+	(+)	41	D-xylose	-	(-)
21	urease	-	-				

[a] + = Positive reaction; - = negative reaction; (+) = weak positive, reaction slightly below detection threshold; (-) = weak negative, reaction slightly above detection threshold
[b] *Enterococcus faecalis* JCM 5803[T]

Description of the Bacteriocin Purification

The purification of the bacteriocin from the culture supernatant of strain MGL-3 was a three-step process starting with the precipitation of total protein from the culture supernatant using ammonium sulfate. Interestingly, the total bacteriocin activity of the crude protein precipitate at 80% saturation of ammonium sulfate appeared to double relative to that of the culture supernatant (Table 2). This is probably because of the removal of media-borne impurities that affected the optimum condition of the bacteriocin to exert its inhibitory activity. Similar observations were noted during the purification of most class IIb bacteriocins (Hu et al., 2010; Nissen-Meyer et al., 1992) but not in other bacteriocin groups (Masuda et al., 2012; Sawa et al., 2009). After purifying the crude protein solution using the prepacked resin OASIS HLB column cartridge, the total activity of the culture supernatant was recovered, suggesting the high efficiency of the purification protocol. Further HPLC purification of the active fraction showed three significant chromatographic peaks but only two peaks manifested bioactivity after spot-on-lawn assay (Figure 2).

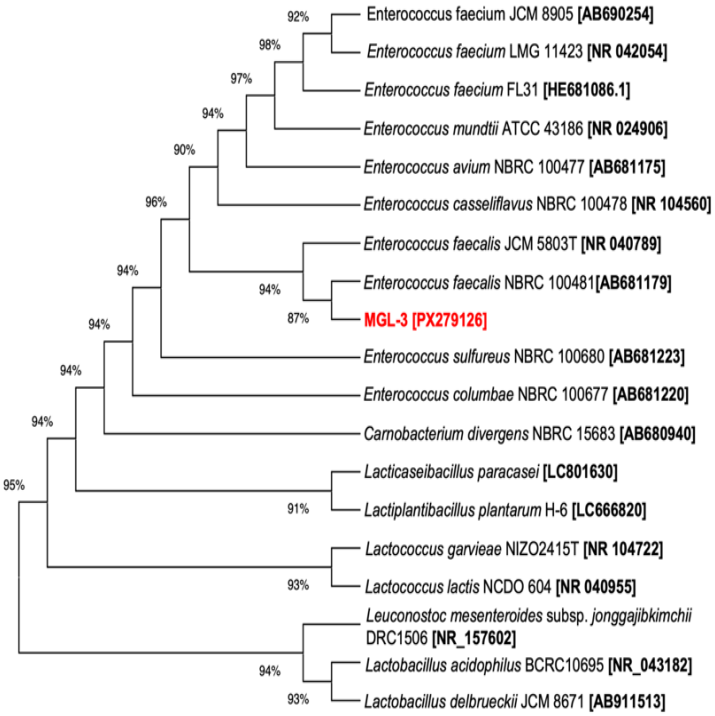

Figure 1. Phylogenetic analysis of the 16S rRNA gene of strain MGL-3 (PX279126) based on the neighbor-joining method in reference to different known species of *Enterococcus* and other common species of lactic acid bacteria.

Table 2. Purification of the bacteriocin of strain MGL-3

Purification Step	Total Volume (mL)	Specific Activity[a] (AU/mg)	Total Protein[b] (mg)	Total Activity (AU)	Purification (fold)
Supernatant	200	10.32	15,500.00	160,000	1.00
$(NH_4)_2SO_4$ Precipitation	50	75.65	4,230.00	320,000	7.33
OASIS® HLB	25	262.30	610.00	160,000	25.41
RP-HPLC	2	339,072.85	0.60	203,444	32,847.68

[a] antimicrobial activity was assayed using spot-on-lawn technique using *L. sakei* ssp. *sakei* JCM 1157^T as indicator strain
[b] estimated from the protein concentration (mg/mL) as quantified using BCA Protein Assay Kit™

Molecular Weight of Each Peptides Component of the Bacteriocin from Strain MGL-3

ESI-TOF MS spectra of the HPLC purified fractions revealed the molecular mass of peptide and to be 3,256.5 Da and 2,728.6 Da, respectively (Figure 3). A query submitted to Bactibase, a dedicated bacteriocin database (Hammami et al., 2010), showed no known bacteriocins with identical molecular mass. Nonetheless, determination of the amino acid sequence and/or the nucleotide sequence of its structural gene is still needed to assess the novelty of these bioactive peptides. In the meantime, these peptides are referred as α and β peptides, comprising the bacteriocin of strain MGL-3.

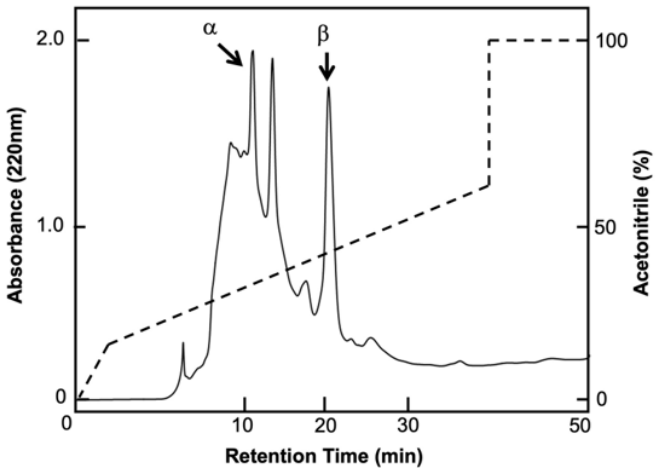

Figure 2. Reversed-phase HPLC chromatogram showing the purification of the peptide components of the bacteriocin from strain MGL-3. The bacteriocin activity was detected in the fractions containing the corresponding peaks indicated by an arrow corresponding the isolation of the and peptides. The broken line indicates the acetonitrile gradient program used during purification.

Bacteriocin Antimicrobial Activity Spectra and Synergistic Activity

The individual and combined bioactivities of the purified peptides against different indicator strains were determined using the spot-on-lawn assay (Table 3). Both individual peptides showed considerably weak bioactivity against most indicator strains. Only the α peptide showed an MIC value less than 10μM against *L. sakei*. Both peptides did not show any bioactivity when tested individually against *Pediococcus pentosaceus* and *L. plantarum*. However, interestingly, when equimolar concentration of these peptides was combined, very potent bioactivity was observed against all indicator strains. The MIC values of the 1:1 mixture of these peptides against all indicator strains were all in the double-digit nanomolar range. Very potent bioactivities of the combination of these peptides against common spoilage microorganisms of vegetable pickles, such as *Bacillus coagulans*, *B. subtilis*, *L. sakei*, *L. plantarum* and *Leuconostoc mesenteroides* (Li et al., 2014; Masuda et al., 2012). The peptide mixture of these peptides showed MIC values of 15nM, 59nM, 7nM, 69nM and 29nM for these spoilage microorganisms, respectively.Furthermore, the FIC indices of these bacteriocinogenic peptides were calculated to all below 0.5 values, demonstrating synergistic activities against almost all indicator strains tested. The observed increase in the potency of these peptides when mixed is typical of two-peptide bacteriocins (Nissen-Meyer et al., 2010). Two-peptide bacteriocins require the presence of both peptides in equal amounts to exert their maximum antibacterial activity (Oppegard et al., 2007). For instance, the two complementary peptides that constitute lactococcin G are active at pico- to nanomolar concentrations when combined, but when tested individually, no significant bioactivity is observed even at concentrations as high as 50μM (Moll et al., 1996).

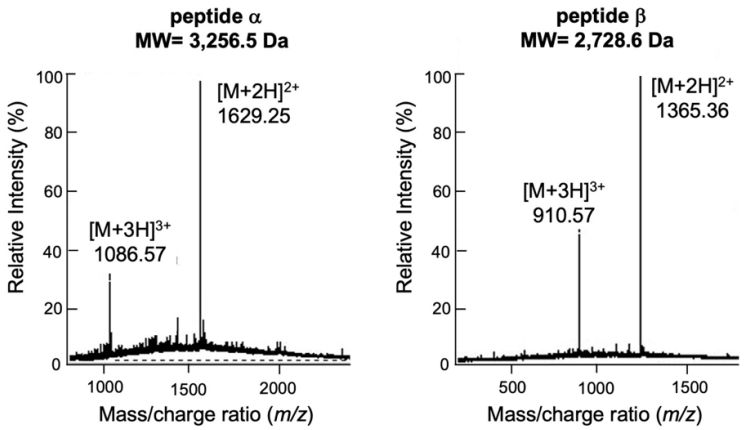

Figure 3. Electrospray ionization time-of-flight mass spectra (ESI-TOF MS) of the purified peptides α and β, a two-synergistic-peptide bacteriocin from strain MGL-3. Multiple charged molecular ion species for each peptide that were detected are indicated. Molecular mass of each peptide component was calculated from the most abundant double-charged ion species for each peptide.

To further demonstrate and visualize the synergistic activity of these peptides, each peptide was spotted next to each other in an agar lawn seeded with the indicator strain. The peptide appeared to have weaker inhibitory activity compared to the peptide against *L. sakei* and did not show noticeable activity against *E. faecalis* in the agar lawn because the spotted solution of 50μM is lower than its MIC value. However, a typical synergistic activity indicated by an oval-shaped zone of inhibition between the spotted peptides was apparent in these agar lawns (Figure 4).

Table 3. Antimicrobial spectra of each peptide and the combined synergistic activity of the bacteriocin component from strain MGL-3

Indicator strains[a]	Minimum Inhibitory Concentration (MIC, μM)[b]			FIC[c]
	α	β	1:1	
Leuconostoc mesenteroides JCM 6124^T	30	-	0.029	NA
Latilactobacillus sakei ssp. *sakei* JCM 1157^T	7.5	15	0.007	0.001
Pediococcus pentosaceus JCM 5885	-	-	0.069	NA
Lactococcus lactis ssp. *lactis* ATCC 19435^T	60	60	0.015	0.001
Bacillus coagulans JCM 2257^T	15	30	0.015	0.002
Lactiplantibacillus plantarum ATCC 14917^T	-	-	0.069	NA
Enterococcus faecalis JCM 5803^T	30	60	0.030	0.002
Listeria innocua ATCC 33090^T	60	-	0.030	NA
Pediococcus dextrinicus JCM 5887^T	7.5	15	0.004	0.001
Bacillus subtilis JCM 1465^T	30	60	0.059	0.003
Enterococcus faecium NKR-5-3	30	60	0.059	0.003
Kocuria rhizophila NBRC 12708	60	60	0.038	0.001

[a] JCM, Japan Collection of Microorganisms, RIKEN, Tsukuba, Japan
ATCC, American Type Culture Collection (Rockville, MD)
NBRC, NITE Biological Resource Center, Chiba, Japan

[b] MIC values were based on the quantified concentration of purified bacteriocin peptide components. Values represent the average from 3 trials that generated exact values, thus standard deviations are not reflected.

[c] The Fractional inhibitory concentration (FIC) was calculated as FIC index = MICα in combination/MICα alone + MICβ in combination/MICβ alone. FIC values of <0.5 indicate synergistic interaction, values between 0.5 and 1.0 indicate additive interaction, and >1 indicate antagonistic interaction.

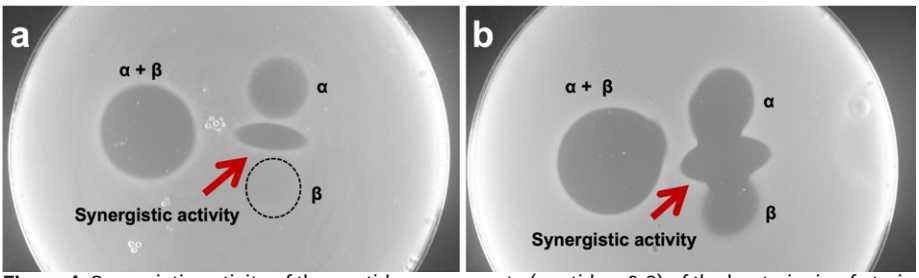

Figure 4. Synergistic activity of the peptide components (peptide α & β) of the bacteriocin of strain MGL-3. A 10L of a 60M pure solution of each peptide was spotted next to each other in an agar lawn seeded with 1% actively growing (a) *Enterococcus faecalis* JCM 5803^{T} and (b) *Latilactobacillus sakei* subsp. *sakei* JCM 1157^{T}as indicator strain. A 1:1 ratio solution containing each peptide component was also spotted in the agar lawn. The synergistic activity of the peptide components in each agar lawn is indicated.

However, it is still unclear if this bacteriocin belongs to class IIb or class I bacteriocins. It should be noted that two-component lantibiotics (class I) bacteriocins have also been reported (Cotter et al., 2006; Willey & van der Donk, 2007). This underlines the importance of solving the amino acid sequence and/or the DNA sequence of its biosynthetic machinery, including its bacteriocin structural genes.

CONCLUSION

In this present study, the identification of strain MGL-3 as *E. faecalis* and the establishment of the purification system, and partial characterization of its two-component bacteriocin are described. The two bioactive peptides of this strain showed synergistic activity against common bacterial contaminants in vegetable pickles, highlighting their potential utility in the industry.

Acknowledgment

The work described here was financially supported in part by the BIOTECH-Core Research Fund (Project Code: 16224). The author would also like to thank the Philippine National Collection of Microorganism (PNCM) for providing access to the BioMérieux® Vitek2® Identification System.

Author Contributions

RHP conducted the experiments and wrote the manuscript.

Funding Source

This study was funded by the BIOTECH Core Research Fund (Project Code: 16224).

Availability of Data and Materials

Data and materials used in this study are available upon request. Genomic data of strain MGL-3 is available at the NCBI Genbank database with the accession number: PX279126.

Ethical Considerations

Experiments described in this study do not require any ethical permit as it did not involve animals and/or human subjects.

Competing Interest

The author is a member of the editorial board of the Annals of Tropical Research.

References

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety, 2*(3), 82–100. https://doi.org/10.1111/j.1541-4337.2003.tb00016.x

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: Safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology, 71*(1), 1–20. http://www.ncbi.nlm.nih.gov/pubmed/11764886

Cotter, P. D., Deegan, L. H., Lawton, E. M., Draper, L. A., O'Connor, P. M., Hill, C., & Ross, R. P. (2006). Complete alanine scanning of the two-component lantibiotic lacticin 3147: Generating a blueprint for rational drug design. *Molecular Microbiology, 62*(3), 735–747. https://doi.org/10.1111/j.1365-2958.2006.05398.x

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology, 3*(10), 777–788. https://doi.org/10.1038/nrmicro1273

Hammami, R., Zouhir, A., Le Lay, C., Ben Hamida, J., & Fliss, I. (2010). BACTIBASE second release: A database and tool platform for bacteriocin characterization. *BMC Microbiology, 10*, Article 22. https://doi.org/10.1186/1471-2180-10-22

Holzapfel, W. H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology, 24*(3), 343–362. http://www.ncbi.nlm.nih.gov/pubmed/7710912

Hu, C. B., Malaphan, W., Zendo, T., Nakayama, J., & Sonomoto, K. (2010). Enterocin X, a novel two-peptide bacteriocin from *Enterococcus faecium* KU-B5, has an antibacterial spectrum entirely different from those of its component peptides. *Applied and Environmental Microbiology, 76*(13), 4542–4545. https://doi.org/10.1128/AEM.02264-09

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiology Reviews, 12*(1), 39–85. http://www.ncbi.nlm.nih.gov/pubmed/8398217

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetics Analysis version 12 for adaptive

and green computing. *Molecular Biology and Evolution, 41*(12),1–9. https://academic.oup.com/mbe/article/41/12/msae263/7930299

Li, K., Lin, K., Li, Z., Zhang, Q., Song, F., Che, Z., Chen, G., & Xiang, W. (2014). Spoilage and pathogenic bacteria associated with spoilage process of Sichuan pickle during the spontaneous fermentation. *Food Science and Technology Research, 20*(4), 899–904. https://doi.org/10.3136/fstr.20.899

Masuda, Y., Zendo, T., Sawa, N., Perez, R. H., Nakayama, J., & Sonomoto, K. (2012). Characterization and identification of weissellicin Y and weissellicin M, novel bacteriocins produced by *Weissella hellenica* QU 13. *Journal of Applied Microbiology, 112*(1), 99–108. https://doi.org/10.1111/j.1365-2672.2011.05180.x

Moll, G., Ubbink-Kok, T., Hildeng-Hauge, H., Nissen-Meyer, J., Nes, I. F., Konings, W. N., & Driessen, A. J. (1996). Lactococcin G is a potassium ion–conducting, two-component bacteriocin. *Journal of Bacteriology, 178*(3), 600–605. http://www.ncbi.nlm.nih.gov/pubmed/8550488

Nissen-Meyer, J., Holo, H., Havarstein, L. S., Sletten, K., & Nes, I. F. (1992). A novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides. *Journal of Bacteriology, 174*(17), 5686–5692. http://www.ncbi.nlm.nih.gov/pubmed/1512201

Nissen-Meyer, J., Oppegard, C., Rogne, P., Haugen, H. S., & Kristiansen, P. E. (2010). Structure and mode of action of the two-peptide (class IIb) bacteriocins. *Probiotics and Antimicrobial Proteins, 2*(1), 52–60. https://doi.org/10.1007/s12602-009-9021-z

Oppegård, C., Rogne, P., Emanuelsen, L., Kristiansen, P. E., Fimland, G., & Nissen-Meyer, J. (2007). The two-peptide class II bacteriocins: Structure, production, and mode of action. *Journal of Molecular Microbiology and Biotechnology, 13*(4), 210–219. https://doi.org/10.1159/000104750

Perez, R. H., Himeno, K., Zendo, T., & Sonomoto, K. (2020). Critical fermentation factors that influence the production of multiple bacteriocins of *Enterococcus faecium* NKR-5-3. *Annals of Tropical Research, 42*(2), 71–84. https://doi.org/10.32945/atr4226.2020

Perez, R. H., Zendo, T., & Sonomoto, K. (2014).Novel bacteriocins from lactic acid bacteria (LAB): Various structures and applications. *Microbial Cell Factories, 13*(Suppl 3). https://doi.org/10.1186/1475-2859-13-S1-S3

Sawa, N., Zendo, T., Kiyofuji, J., Fujita, K., Himeno, K., Nakayama, J., & Sonomoto, K. (2009). Identification and characterization of lactocyclicin Q, a novel cyclic bacteriocin produced by *Lactococcus* sp. strain QU 12. *Applied and Environmental Microbiology, 75*(6), 1552–1558. https://doi.org/10.1128/AEM.02299-08

Tajima, K., Arai, S., Ogata, K., Nagamine, T., Matsui, H., Nakamura, M., Aminov, R. I., & Benno, Y. (2000). Rumen bacterial community transition during adaptation to a high-grain diet. *Anaerobe, 6*(5), 273–284. https://doi.org/10.1006/anae.2000.0353

U.S. Food and Drug Administration. (1988). *Nisin preparation: Affirmation of GRAS status as a direct human food ingredient* (21 CFR Part 184). *Federal Register, 53*, 11247–11251. https://archives.federalregister.gov/issue_slice/1988/4/6/11245-11253.pdf

Willey, J. M., & van der Donk, W. A. (2007). Lantibiotics: Peptides of diverse structure and function. *Annual Review of Microbiology, 61*, 477–501. https://doi.org/10.1146/annurev.micro.61.080706.093501