

Characterization of the Antimicrobial Substances Produced by *Nibribacter radioresistens*

(Pencirian Bahan Antimikrob yang Dihasilkan oleh *Nibribacter radioresistens*)

SAM WOONG KIM, YEON JO HA, SANG WAN GAL, KYU PIL LEE, KYU HO BANG, MYUNG-SUK KANG, JOO-HONG YEO,
HEE-SUN YANG, SEUNG-HO JEON & WOO YOUNG BANG*

ABSTRACT

This study characterized the antimicrobial substances produced by the radiation-resistant bacterium Nibribacter radioresistens. The antimicrobial substances showed activity against Salmonella Gallinarum, pathogenic Escherichia coli, Bacillus cereus, Streptococcus iniae, and Saccharomyces cerevisiae. The substances showed higher activity against Gram-positive bacteria than against Gram-negative bacteria and yeast. N. radioresistens showed the best growth rate in LB liquid medium at 37°C; however, production of the antimicrobial substances was not associated with growth. Since the activity of the antimicrobial substances was affected by proteinase K and EDTA, the substances were presumed to be antimicrobial peptides (AMPs). The antimicrobial substances produced by N. radioresistens were unstable at higher temperatures and in acidic and basic pH ranges, and most of the activity was attributed to either low (<3 kDa) or high molecular weight (>30 kDa) molecules. When S. Gallinarum was treated with the antimicrobial substances, the cell destruction was acted on the cell envelope. Therefore, we concluded that N. radioresistens produces broad-spectrum and very unstable antimicrobial substances that mostly consist of low- and high-molecular weight peptides.

Keywords: AMPs; antimicrobial activity; growth curve; Nibribacter radioresistens; protease; radiation-resistant bacteria

ABSTRAK

Kajian ini dicirikan bahan antimikrob yang dihasilkan oleh bakteria Nibribacter radioresistens. Bahan antimikrob menunjukkan aktiviti menentang Salmonella Gallinarum, patogen Escherichia coli, Bacillus cereus, Streptococcus iniae dan Saccharomyces cerevisiae. Bahan yang menunjukkan aktiviti yang lebih tinggi terhadap bakteria gram-positif berbanding dengan bakteria gram-negatif dan yis. N. radioresistens menunjukkan kadar pertumbuhan terbaik dalam cecair medium lb pada 37°C; walau bagaimanapun, pengeluaran bahan antimikrob tidak dikaitkan dengan pertumbuhan. Oleh kerana aktiviti bahan antimikrob terjejas oleh proteinase k dan edta, bahan tersebut dianggap sebagai antimikrob peptida (AMPs). Bahan antimikrob yang dihasilkan oleh N. radioresistens tidak stabil pada suhu yang lebih tinggi dan berada dalam julat berasid dan ph asas, dan sebahagian besar aktiviti itu disebabkan sama ada rendah (<3 kda) atau berat molekul tinggi (>30 kda). Apabila S. Gallinarum dirawat dengan bahan antimikrob, pemusnahan sel telah berlaku pada sampul sel. Oleh itu, kami menyimpulkan bahawa N. radioresistens menghasilkan bahan antimikrob yang luas dan sangat tidak stabil yang kebanyakannya terdiri daripada peptida berat molekul rendah dan tinggi.

Kata kunci: Aktiviti antimikrob; AMPs; bakteria rintangan-sinaran; keluk pertumbuhan; Nibribacter radioresisten; sprotease

INTRODUCTION

Many microorganisms have adapted to survival in extreme environments, such as high/low temperature, extreme pH, salinity, osmotic pressure, and radiation (Dopson et al. 2016; Raddadi et al. 2015; Sarmiento et al. 2015). Microorganisms in extreme environments have various resistance mechanisms to survive in extreme environments, and produce unique product for industrial application (Cabreera & Blamey 2018; Chen & Jiang 2018). *Nibribacter radioresistens* was obtained during a search for microorganisms in unique environments in Korea (NIBR 2016; Sathiyaraj et al. 2018). *Nibribacter* is a genus of Gram-negative bacteria in the family Hymenobacteraceae. The bacterial colony grows on R2A agar with reddish

pigment after 24 h at 25°C. The cell is a short stick shape with size 1.8-3.6 µm in length (Kang et al. 2013; Lin et al. 2018). The genus *Nibribacter* was first reported in 2013, and to date, three species have been published (Kang et al. 2013; Lin et al. 2018; Sathiyaraj et al. 2018). *N. koreensis* is a representative species of this genus, and *N. radioresistens* is a newer species that is radiation resistant (NIBR 2016; Sathiyaraj et al. 2018).

Most living organisms generate substances to protect themselves from other organisms. Typically, these substances are either antibiotics or peptide-based antimicrobial products. Peptide-based antimicrobial substances, which are called antimicrobial peptides (AMPs), have been identified in most organisms (Bulet & Stocklin

2005). AMPs are generally composed of cationic substances in low molecular weight, and perform their functions by destroying cell membrane of microorganism (Bulet & Stocklin 2005). The function of AMPs is not limited to direct killing of microorganisms, but related with action of immunomodulation and with regulation of endosymbiotic bacteria (Easton et al. 2009; Login et al. 2011). AMPs are classified within three groups according to structure and nonspecific richness of specific amino acids: Alpha-helical peptides lacking cysteine residues; beta-sheet globular structure stabilized by intramolecular disulfide bridges; and rich peptides to maintain a large proportion of nonspecific proline or glycine residues.

Many microorganisms produce potent AMPs called bacteriocin (Azevedo et al. 2015; Walsh et al. 2015). Although most AMPs are low molecular weight peptides composed of 20–40 amino acids, some are high molecular weight molecules (Dimopoulos et al. 1997; Reddy et al. 2004; Wang 2013). AMPs can be produced by either the cleavage of proteins synthesized from ribosomes or by non-ribosomal mechanisms (Mousa & Raizada 2015; Tajbakhsh et al. 2017). Although AMPs are susceptible to degradation by proteases, AMPs that include D-amino acids or cyclic amino acids are resistant to protease degradation (Kajimura & Kaneda 1997; Schwarzer et al. 2003).

In this study, The *N. radioresistens* metabolite was extracted and its antimicrobial activity was evaluated. In addition, we studied physicochemical properties for the antimicrobial substances.

MATERIALS AND METHODS

MEDIA AND BACTERIAL STRAINS

The media used for *N. radioresistens* culture in this study were R2A (Reasoner's 2A), TSB (tryptic soy broth), and LB (Luria-Bertani broth) (Sigma-Aldrich, USA). Solid medium was prepared by adding 1.5% agar. *Salmonella* Gallinarum HJL462, *Escherichia coli* JOL418, and *Streptococcus iniae* S186 were obtained from Dr. Jin Hur (Chonbuk National University, Iksan, South Korea) and Dr. Tae Sung Jung (Gyeongsang National University, South Korea). *Bacillus cereus* (ATCC 1178) and *Saccharomyces cerevisiae* (KCTC 27134) were obtained from the Korean Collection for Type Cultures (KCTC). *S. Gallinarum*, *E. coli*, *S. iniae*, and *B. cereus* were cultured at 37°C, and *S. cerevisiae* was cultured at 30°C.

ASSAYS OF ANTIMICROBIAL ACTIVITY

N. radioresistens were cultured in R2A, TSB and LB broth for 48 h at 25, 30, and 37°C. The medium was then centrifuged at 2,000 × g for 20 min. The resulting supernatant was collected, filtered through Whatman No.1 filter paper, and sterilized by filtration through a 0.22 µm filter. The sterilized solution was either used directly for

analysis of antimicrobial activity or fractionated and stored at -80°C until use.

The antimicrobial activity on agar plates was done by some modification according to dilution assay of Wiegand et al. (2008). Briefly, the medium was punched with a 7-mm cork borer, and the susceptibility test strain was spread on the plate with 10⁶ CFU/mL. Then, different amounts of *N. radioresistens* test liquid (0, 600, 900, and 1,200 µg/mL) were added to the punched holes. The clear zone formation was observed after each plate was incubated accordingly for 48 h at 25°C.

To examine the antimicrobial activity on a microtiter plate (Ha et al. 2017), the serially diluted cell free supernatant was mixed with the diluted susceptibility test strain (10⁶ CFU/mL) and fresh medium in each well. Then, the microtiter plate was incubated at the appropriate temperature for 48 h, and antimicrobial activity was evaluated by measuring the absorbance at 600 nm and determining the viable cell counts.

EXAMINATION OF THE GROWTH DEPENDENCE OF ANTIBACTERIAL ACTIVITY

Growth performance and antimicrobial activity in different media (R2A, TSB, and LB) were compared during incubation at 25°C for 48 h. Growth curves in LB medium were generated at 25, 30, and 37°C, with sampling and absorbance (at 600 nm) measurements every 6 h. Growth curves were generated in LB medium at 37°C over 54 h, with sampling and absorbance (at 600 nm) measurements every 6 h. The antimicrobial activities of the culture broth sampled during the growth curve experiments were analyzed by a microtiter plate assay against *S. Gallinarum* and *B. cereus*.

EFFECTS OF PROTEINASE K AND EDTA TREATMENT

The effects of proteinase K treatment were assessed by adding proteinase K (20 mg/mL stock), at a concentration of 100 µg/mL, to *N. radioresistens* samples in a microtiter plate assay. Then, antimicrobial activity against *S. Gallinarum* was analyzed by adding the test strain to the microtiter plate at an initial concentration of 10⁸ CFU/mL, and the supernatant was applied at 1.4 mg peptides/mL of corresponding to theoretical MIC value in a 10⁶ CFU/mL. Then, the microtiter plate was incubated for 24 h at 37°C, and antimicrobial activity was examined by measuring the absorbance at 600 nm.

The effects of EDTA on antimicrobial activity were also assessed in a microtiter plate assay by adding EDTA at 0, 5, 10, 15, 20, 25, and 30 mM in an assay with *S. Gallinarum* at 10⁸ CFU/mL, and the supernatant was applied at 1.4 mg/mL of corresponding to theoretical MIC value a 10⁶ CFU/mL. Then, the microtiter plate was incubated for 24 h at 37°C, and antimicrobial activity was examined by measuring the absorbance at 600 nm.

EXAMINATION OF THE PHYSICO-CHEMICAL PROPERTIES OF THE ANTIMICROBIAL MOLECULES

The heat stability of the antimicrobial substances was assessed by measuring their antimicrobial activity after incubation at 30, 40, 50, 60, 70, and 80°C for 10 min and cooling in an ice bath. Then, the treated solutions were mixed with the diluted susceptibility test strain and fresh medium for a microtiter plate assay. The microtiter plate was incubated for 24 h at 37°C, and antimicrobial activity was examined by measuring the absorbance at 600 nm. The pH stability was measured by a microtiter plate assay after adjusting the supernatant fluid to different pH values such as 2, 4, 6, 7, 8, 10, and 12.

To evaluate the antimicrobial activity of culture supernatant fractions of different molecular weights, the culture supernatant was separated into four fractions: <3 kDa, 3-10 kDa, 10-30 kDa, and >30 kDa, by Centricon (Millipore, USA). Then, the antimicrobial activity of the fractionated samples was evaluated in a microtiter plate assay.

To analyze the chemical properties of the antimicrobial substances, supernatant samples were adjusted to 30%, 50%, and 70% methanol by adding 100% methanol, incubated at 4°C for 1 h, and then centrifuged at 2,000 × g for 20 min. The supernatant was collected, completely dried by concentration under reduced pressure, and suspended in distilled water to generate a 10-fold concentrate of the initial supernatant. Then, the antimicrobial activity of the treated samples was evaluated in a microtiter plate assay.

SCANNING ELECTRON MICROSCOPY (SEM)

S. Gallinarum was incubated with cell-free supernatant from *N. radioresistens* culture for 0, 24, and 36 h. The treated *S. Gallinarum* cells were fixed with one volume of 2.5% glutaraldehyde (Sigma-Aldrich) for 24 h at 4°C. Then, the samples were rinsed with sterile PBS buffer thrice and sequentially dehydrated with graded ethanol (30%, 50%, 70%, 80%, 90%, and 100% [v/v]; 15 min incubation for each concentration). Finally, the samples were dried at room temperature and sputter-coated with gold for SEM.

STATISTICAL ANALYSIS

The collected data were analyzed using the PROC ANOVA procedure of the SAS program (ver. 9.2; SAS Institute Inc, Cary, NC, USA). Mean values that differed at the level of 5% significance were verified using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

THE SUPERNATANT OF *N. RADIORESISTENS* CULTURES HAS ANTIBACTERIAL ACTIVITY

In this study, we examined the antimicrobial activity of substances produced by *N. radioresistens*. As shown in Figure 1, the culture supernatant of *N. radioresistens* showed antimicrobial activities against Gram-positive

bacteria, and Gram-negative bacteria, but very low activity against yeast. The Lethal doses 50 (LD50) of the culture supernatant were 925.7±8.06, 1,062.6±2.54, 732.0±3.61, 920.8±20.42, 3,909.8±78.3 µg/mL for *S. Gallinarum*, *E. coli*, *B. cereus*, *S. iniae*, and *S. cerevisiae*, respectively. The supernatant showed stronger activity against Gram-positive bacteria than against Gram-negative bacteria and yeast, and showed the highest activity against *S. iniae*. Thus, the antimicrobial substances in the supernatant appeared to have broad spectrum activity, but higher activity against Gram-positive bacteria.

Antibiotic is a commonly produced by microorganisms as antimicrobial substances that confers a competitive advantage against other bacteria (reviewed by Ueda & Beppu 2017). Currently, the discovery of new antibiotics in microbial culture is very difficult because of limiting the chance of new discovery (Ueda & Beppu 2017). Therefore, there is a necessity to isolate new microorganisms and to continue with new attempts. In one aspect of the study, the antimicrobial activity against *N. radioresistens*, a newly isolated microorganism, was examined in this study. As a result, it was observed that a broad range of antimicrobial activity is present in the *N. radioresistens* supernatant. The antimicrobial activity observed in this study may be that of a single substance with broad activity or multiple antimicrobial substances with various activities.

ANTIBACTERIAL ACTIVITY IS NOT ASSOCIATED WITH GROWTH, BUT IS DEPENDENT OF THE SUPERNATANT CONCENTRATION

In general, *Nibribacter* is cultured in R2A medium at 25°C (Kang et al. 2013; Sathiyaraj et al. 2018). We evaluated the growth of *N. radioresistens* in different media and observed that *N. radioresistens* showed better growth rate in LB broth than in R2A and TSB, although the antibacterial activity produced in these media were similar pattern with growth rate (data not shown). Therefore, for subsequent analyses of antimicrobial activity, *N. radioresistens* was grown in LB medium. The *N. radioresistens* growth curves showed similar patterns at 30°C and 37°C, and the bacteria grew faster at 30°C and 37°C than at 25°C (Figure 2(A)). When incubated at 25°C, stationary phase was reached at 48 h, whereas when incubated at 30°C and 37°C, stationary phase was reached within 24 h. Incubation at 37°C decreased the time to reach exponential phase compared to that at 30°C. The antimicrobial activity levels in the supernatant of cultures after growth for 24 h at the different temperatures were similar (data not shown).

Antimicrobial activity was monitored during incubation at 37°C, and it reached a maximum at the end of exponential phase and was maintained throughout the experimental period (Figure 2(B)). Although the tendency of secondary metabolites to rapidly decline after production was not observed in this study, the production of these antimicrobial substances was typical of secondary metabolites (Demain 1998). The bacteriocin

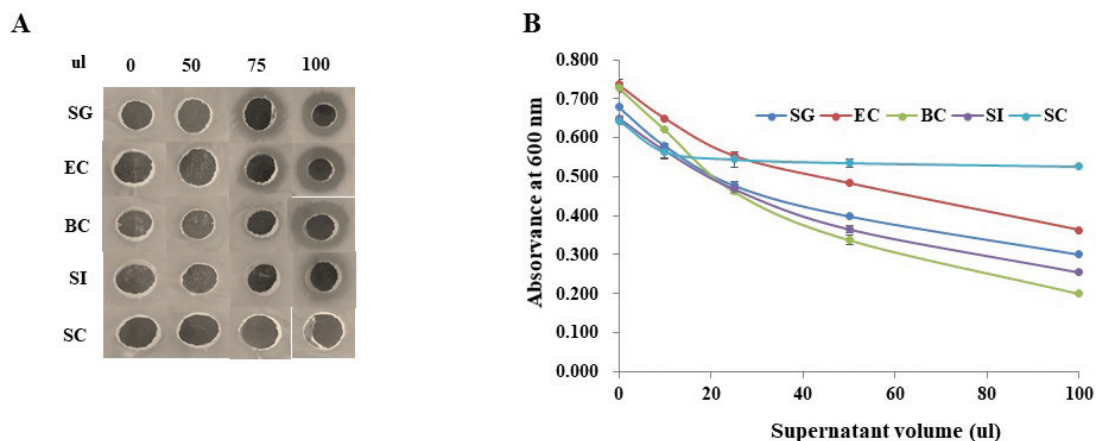


FIGURE 1. Assays of the antibacterial activity in the culture supernatant of *Nibribacter radioresistens*. Assays on agar plates (A) and in microtiter plates (B). Supernatants collected from *N. radioresistens* cultures were assessed for antibacterial activities. SG, *Salmonella Gallinarum*; EC, pathogenic *Escherichia coli*; BC, *Bacillus cereus*; SI, *Streptococcus iniae*; SC, *Saccharomyces cerevisiae*

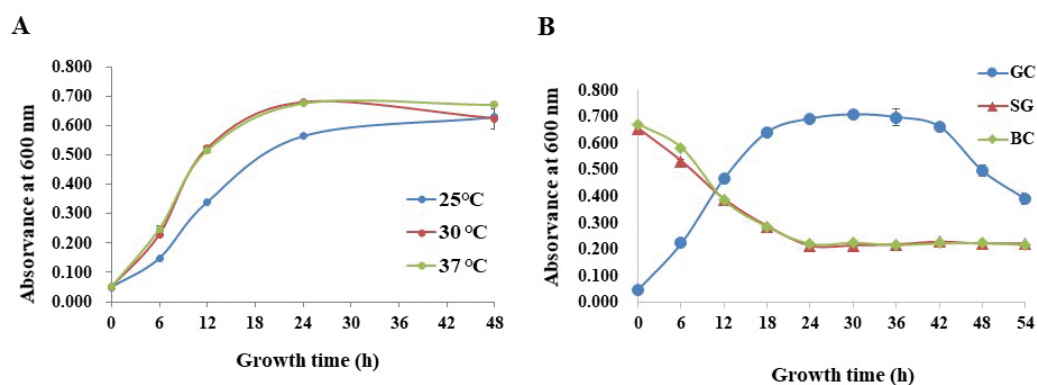


FIGURE 2. Growth curves and antibacterial activities in supernatant of *N. radioresistens*. Assays of the growth temperature dependence (A) and growth phase dependence (at 37°C) of antibacterial activity (B). *N. radioresistens* was grown at the indicated temperatures, and antibacterial assays were performed in a microtiter plate with culture supernatants collected during growth. GC, growth curve for *N. radioresistens*; SG, *Salmonella Gallinarum*; BC, *Bacillus cereus*

produced by *Weissella confusa* showed a similar pattern to the antimicrobial substances in this study (Goh & Philip 2015). The antimicrobial activity was dependent on the amount of susceptibility test strain, which was inversely proportional to the concentration of *S. Gallinarum* and *B. cereus* (data not shown). Therefore, the activity of these antimicrobial substances is dependent on the concentration of the substances themselves as well as the target microbes.

THE CHARACTERISTICS OF THE ANTIBACTERIAL SUBSTANCES IN THE SUPERNATANT OF *N. RADIORESISTENS* CULTURE ARE SIMILAR TO THOSE OF PEPTIDES

Based on the results of the experiment testing the relationship between antimicrobial activity and growth, the possibility of antimicrobial secondary metabolites could not be ruled out. Therefore, the culture supernatant was treated with proteinase K and EDTA to determine whether the antimicrobial substance was derived from a metabolite or a peptide. Proteinase K treatment decreased activity

by 81.2% compared to that of the control (Figure 3(A)). Bacteriocin appears to be severely degraded by protease treatment (Kaur & Tiwari 2018). In this study, since the antimicrobial activity was mostly lost by treatment with proteinase K, this suggests that most of the antibacterial activity is derived from peptide-based substances.

It is well-known that the proteases contained in culture supernatant can inhibit the antimicrobial activities of AMPs (Ha et al. 2017). Since most of the antimicrobial substances contained in the supernatant of this culture are likely AMPs, we analyzed the effect of EDTA, a protease inhibitor, on antimicrobial activity. The results showed that EDTA enhanced antimicrobial activity. Specifically, the addition of 20 mM EDTA enhanced antimicrobial activity by 39.9% compared to that of the control (0 mM, only added with culture supernatant) (Figure 3(B)). This result was similar to the results obtained following treatment of culture supernatant from an *E. coli* strain producing mastoparan V1 with various protease inhibitors (Ha et al. 2017).

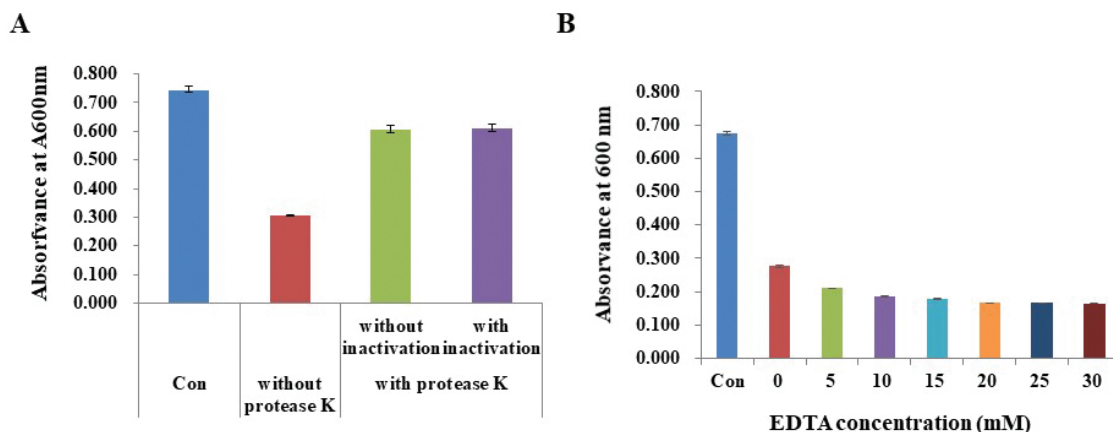
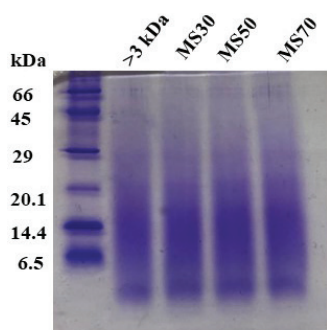


FIGURE 3. Assays of the effects of proteinase K and EDTA on antibacterial activity in the supernatant of *N. radioresistens*. Culture supernatant was treated with proteinase K (A) and the protease inhibitor EDTA (B). The test bacterial strain, *S. Gallinarum*, was added at 10^8 CFU/mL in A and B, respectively

Suppl. 3. SDS-PAGE of each fraction according to methanol fractionation



Each fraction was loaded on 15% SDS-PAGE with 20 mg

THE ANTIBACTERIAL SUBSTANCES ARE MOSTLY UNSTABLE PEPTIDES

The *N. radioresistens*-derived antimicrobial substances were stable at temperatures below 40°C, but decreased sharply above 50°C (Figure 4(A)). In addition, the

substances were only stable around neutral pH, and were very unstable outside this range (Figure 4(B)). The bacteriocins produced by lactic acid bacteria maintained temperature stability up to around 60°C, and confirm stability in most pH ranges (Garsa et al. 2014). When compared with lactic acid bacteria-derived bacteriocins, it is suggested that *N. radioresistens*-derived antimicrobial substances are stable only in temperature and pH of specific regions.

The molecular weights and chemical properties of the *N. radioresistens*-derived antimicrobial substances were examined by molecular weight-based separation and methanol fractionation. Antimicrobial activity was detected in all size fractions (Figure 5(A)). However, higher activities were observed in the fractions containing molecules <3 kDa and >30 kDa. Therefore, based on this and the results of the proteinase K treatment, we concluded that the antibacterial activity is due to the presence of peptide-based antibacterial substances of both low and high molecular weights.

Antimicrobial activity was detected in all methanol fractions, but the highest activity was observed in the 70%

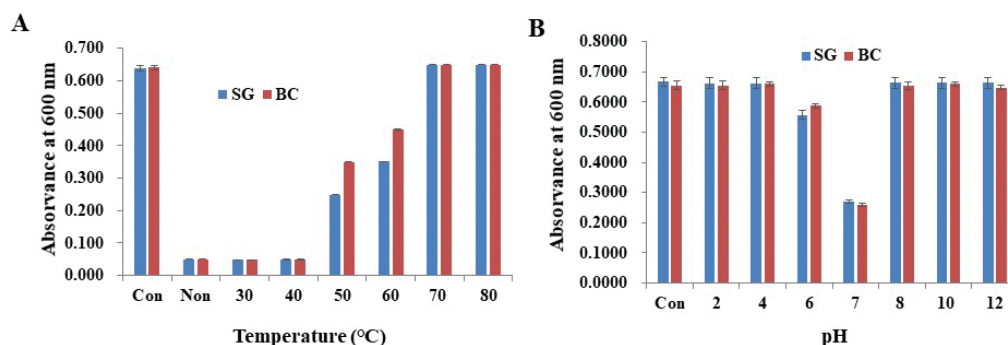


FIGURE 4. Assays of the thermostability (A) and pH stability (B) of the antibacterial substances. The thermostability experiment was performed by incubation at the indicated temperature for 10 min. For the pH experiment, the pH of the supernatant was adjusted to the indicated values. Non, non-treated; SG, *Salmonella Gallinarum*; BC, *Bacillus cereus*. Bars with different letters within each examined component differ significantly at $P < 0.05$

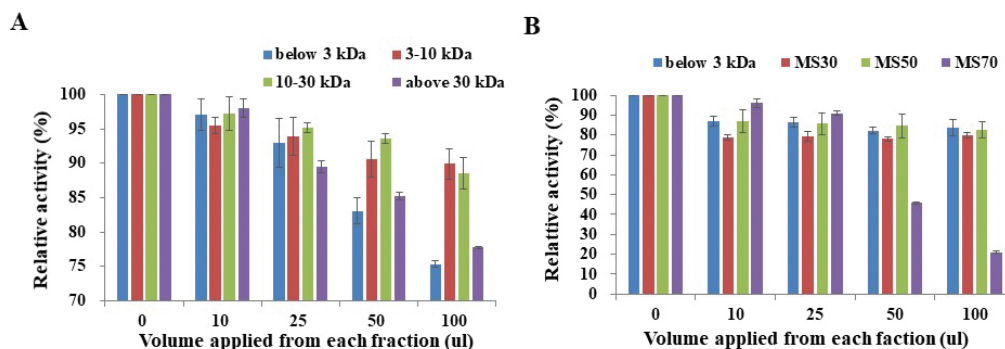


FIGURE 5. Physicochemical properties of the antibacterial substances. Antibacterial activity of molecular weight (A) and methanol (B) fractions. The supernatant was separated by molecular weight as indicated. Methanol (100%) was added to the supernatant to various final concentrations. Then, an antibacterial assay was performed with *S. Gallinarum*. MS0, untreated; MS30, 50, and 70 indicate the supernatants of 30%, 50%, and 70% methanol fractionations, respectively. Bars with different letters within each examined component differ significantly at $P < 0.05$

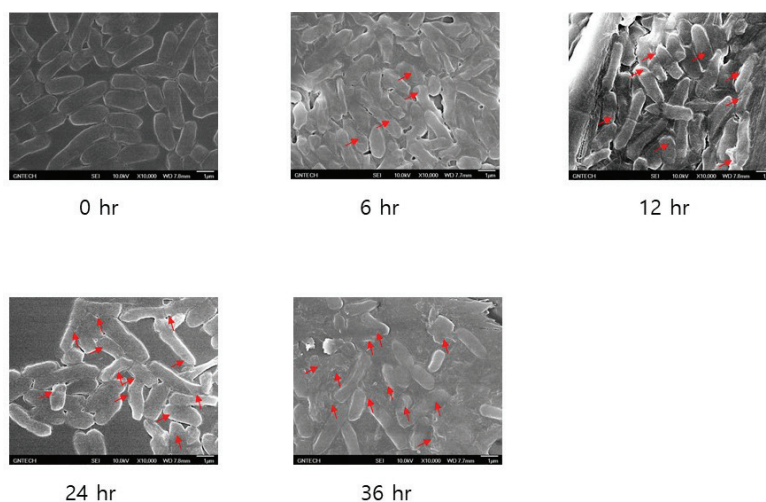


FIGURE 6. SEM observations. *S. Gallinarum* was treated with *N. radioresistens* culture supernatant and observed over time. The red arrows indicate pores produced in the *S. Gallinarum* cell wall

methanol supernatant (Figure 5(B)). Therefore, it was thought that most of the antimicrobial substances exhibit somewhat low hydrophilicity. Most AMPs have an amino acid composition with 50% hydrophobicity (Wang 2013). Therefore, as a result of the methanol fractionation, it is estimated that the antimicrobial substances shown in this study may have amino acid composition of nearly 50% hydrophobicity.

The effects of the AMPs predicted in the present study on *Salmonella* were observed by scanning electron microscopy (SEM). The experiment showed that the antimicrobial substances were acted on the surface of cell, which induced lysis (Figure 6). In addition, as treatment time elapsed, cell disruption was found to increase. Therefore, we suggest that the antimicrobial substances induce bacterial lysis by acting on the cell walls of the bacteria.

Our results suggested that most of the antimicrobial activity produced by *N. radioresistens* is due to low and high molecular weight AMPs that are very unstable in high heat and at non-neutral pH.

ACKNOWLEDGEMENTS

This work was supported by a grant (NIBR201827201) from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea. Both authors Sam Woong Kim and Yeon Jo Ha contributed equally to this work.

REFERENCES

- Azevedo, A.C., Bento, C.B., Ruiz, J.C., Queiroz, M.V. & Mantovani, H.C. 2015. Distribution and genetic diversity of bacteriocin gene clusters in rumen microbial genomes. *Appl. Environ. Microbiol.* 81: 7290-7304.
- Bulet, P. & Stocklin, R. 2005. Insect antimicrobial peptides: structures, properties and gene regulation. *Protein Pept. Lett.* 12: 3-11.
- Cabrera, M.A. & Blamey, J.M. 2018. Biotechnological applications of archaeal enzymes from extreme environments. *Biol Res.* 51: 37.
- Chen, G.Q. & Jiang, X.R. 2018. Next generation industrial biotechnology based on extremophilic bacteria. *Curr. Opin. Biotechnol.* 50: 94100.

- Demain, A.L. 1998. Induction of microbial secondary metabolism. *Int. Microbiol.* 1: 259-264.
- Dimopoulos, G., Richman, A., Müller, H.M. & Kafatos, F.C. 1997. Molecular immune responses of the mosquito *Anopheles gambiae* to bacteria and malaria parasites. *Proc. Natl. Acad. Sci. USA.* 94: 11508-11513.
- Dopson, M., Ni, G. & Sleutels, T.H. 2016. Possibilities for extremophilic microorganisms in microbial electrochemical systems. *FEMS Microbiol. Rev.* 40: 164-181.
- Easton, D.M., Nijnik, A., Mayer, M.L. & Hancock, R.E. 2009. Potential of immunomodulatory host defense peptides as novel anti-infectives. *Trends Biotechnol.* 27: 582-590.
- Garsa, A.K., Kumariya, R., Sood, S.K., Kumar, A. & Kapila, S. 2014. Bacteriocin production and different strategies for their recovery and purification. *Probiotics Antimicrob. Proteins* 6: 47-58.
- Goh, H.F. & Philip, K. 2015. Purification and characterization of bacteriocin produced by *Weissella confusa* A3 of dairy origin. *PLoS ONE* 10: e0140434.
- Ha, Y.J., Kim, S.W., Lee, C.W., Bae, C.H., Yeo, J.H., Kim, I.S., Gal, S.W., Hur, J., Jung, H.K., Kim, M.J. & Bang, W.Y. 2017. Anti-*Salmonella* activity modulation of mastoparan V1-a wasp venom toxin-using protease inhibitors, and its efficient production via an *Escherichia coli* secretion system. *Toxins* 9: pii: E321.
- Kajimura, Y. & Kaneda, M. 1997. Fusaricidins B, C and D, new depsipeptide antibiotics produced by *Bacillus polymyxa* KT-8: Isolation, structure elucidation and biological activity. *J Antibiot.* 50: 220-228.
- Kang, J.Y., Chun, J. & Jahng, K.Y. 2013. *Nibribacter koreensis* gen. nov., sp. nov., isolated from estuarine water. *Int. J. Syst. Evol. Microbiol.* 63: 4663-4668.
- Kaur, R. & Tiwari, S.K. 2018. Membrane-acting bacteriocin purified from a soil isolate *Pediococcus pentosaceus* LB44 shows broad host-range. *Biochem. Biophys. Res. Commun.* 498: 810-816.
- Lin, P., Yan, Z.F., Li, C.T., Kook, M. & Yi, T.H. 2018. *Nibribacter flagellatus* sp. nov., isolated from rhizosphere of *Hibiscus syriacus* and emended description of the genus *Nibribacter*. *Antonie Van Leeuwenhoek.* 111: 1777-1784.
- Login, F.H., Balmand, S., Vallier, A., Vincent-Monégat, C., Vigneron, A., Weiss-Gayet, M., Rochat, D. & Heddi, A. 2011. Antimicrobial peptides keep insect endosymbionts under control. *Science* 334: 362-365.
- Mousa, W.K. & Raizada, M.N. 2015. Biodiversity of genes encoding anti-microbial traits within plant associated microbes. *Front Plant Sci.* 16: 231.
- NIBR. 2016. *Acquisition and Characterization of Extremophiles (II)*. Microorganism Resources Division of Biological Resources Research Department.
- Raddadi, N., Cherif, A., Daffonchio, D., Neifar, M. & Fava, F. 2015. Biotechnological applications of extremophiles, extremozymes and extremolytes. *Appl. Microbiol. Biotechnol.* 99: 7907-7913.
- Reddy, K.V., Yedery, R.D. & Aranha, C. 2004. Antimicrobial peptides: Premises and promises. *Int. J. Antimicrob. Agents* 24: 536-547.
- Sarmiento, F., Peralta, R. & Blamey, J.M. 2015. Cold and hot extremozymes: Industrial relevance and current trends. *Front Bioeng. Biotechnol.* 3: 148.
- Sathiyaraj, G., Kim, M.K., Kim, J.Y., Kim, S.J., Jang, J.H., Maeng, S.H., Kang, M.S. & Srinivasan, S. 2018. Complete genome sequence of *Nibribacter radioresistens* DG15C, a radiation resistant bacterium. *Mol. Cell Toxicol.* 14: 323-328.
- Schwarzer, D., Finking, R. & Marahiel, M.A. 2003. Nonribosomal peptides: From genes to products. *Nat. Prod. Rep.* 20: 275-287.
- Tajbakhsh, M., Karimi, A., Fallah, F. & Akhavan, M.M. 2017. Overview of ribosomal and non-ribosomal antimicrobial peptides produced by Gram positive bacteria. *Cell. Mol. Biol.* 63: 20-32.
- Walsh, C.J., Guinane, C.M., Hill, C., Ross, R.P., O'Toole, P.W. & Cotter, P.D. 2015. *In silico* identification of bacteriocin gene clusters in the gastrointestinal tract, based on the Human Microbiome Project's reference genome database. *BMC Microbiol.* 15: 183.
- Wang, G. 2013. Database-guided discovery of potent peptides to combat HIV-1 or superbugs. *Pharmaceuticals* 6: 728-758.
- Wiegand, I., Hilpert, K. & Hancock, R.E. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* 3: 163-175.
- Sam Woong Kim, Yeon Jo Ha, Sang Wan Gal & Kyu Ho Bang
Gene Analysis Center
Gyeongnam National University of Science & Technology
Jinju 52725
Republic of Korea
- Kyu Pil Lee
Laboratory of Physiology
College of Veterinary Medicine
Chungnam National University
Daejeon 34134
Republic of Korea
- Myung-Suk Kang, Joo-Hong Yeo, Hee-Sun Yang & Woo Young Bang*
National Institute of Biological Resources (NIBR)
Environmental Research Complex
Incheon 22689
Republic of Korea
- Seung-Ho Jeon
Suncheon National University
255, Jungang-ro, Suncheon-si, 57922
Republic of Korea

*Corresponding author; email: wybang@korea.kr

Received: 14 December 2018

Accepted: 16 September 2019