https://dx.doi.org/10.4314/dujopas.v10i4b.2
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 4b December 2024*

Biophysical Characterization of Bacteriocin from *Weissella cibaria* man1

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Abstract

A huge global public health concern is the rise of bacteria and other microorganisms that are resistant to the vast majority of antibiotics used today in healthcare settings. In 2020, the most recent worldwide disease survey found that antibiotic-resistant diseases were responsible for half a million annual deaths. If present trends persist, this number is expected to exceed 10 million by 2050. Weissella cibaria bacteriocins could be the answer to this conundrum. To make its potential usage as a replacement for traditional antibiotics a reality in the future however, knowledge of its biophysical properties is crucial. Consequently, a study aimed at determining the biophysical characteristics of bacteriocin from Weissella cibaria man1 was carried out. Agar well diffusion assay was used to determine antimicrobial activity. The antibacterial activity of bacteriocin from Weissella cibaria man1 was significantly influenced by temperature in a time-dependent fashion. However, the presence of residual inhibitory activity even at 100^{0}C indicates thermal stability of the bacteriocin. Bacteriocin from Weissella cibaria man1 had higher antimicrobial activity at low pH values (2 and 4) than at high pH values (6, 8). Moreover, this bacteriocin displayed resistance to treatment with Tween 80 and Sodium dodecyl sulfate indicating its chemical stability. These discoveries will facilitate the future industrial and clinical applications of bacteriocin from Weissella cibaria man1.

Keywords: Bacteriocin, Biophysical characteristics, pH stability, Thermal stability, *Weissella cibaria* man1,

INTRODUCTION

The development of resistance to majority of antibiotics presently used in clinical settings constitutes a major public health concern on a worldwide scale (Darby *et al.*, 2023). Based on the latest global disease survey in the year 2020, the number of people dying each year from infections that are resistant to antibiotics reaches 500,000. This figure is projected to surpass 10 million by the year 2050, if current trends continue (Wayah *et al.*, 2024). The health consequences of infectious diseases will return to those of the pre-antimicrobial era unless immediate measures are adopted. To make matters worse, illnesses that are resistant to antibiotics would cost more than $100 trillion by the year 2050 (Denku *et al.*, 2022). In light of

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this, the World Health Organization is urging scientists to focus on creating new antimicrobials.

Worldwide, bacteriocins are being regarded as potential substitutes for conventional antibiotics (Cheruvari and Kammara, 2024). Bacteriocins are peptides that are produced by the ribosome. They effectively combat their intended microbes through unique mechanisms that prevent the evolution of resistance, and they are widely believed to be safe for human consumption (Pepi *et al.*, 2024). Their chemical, pH, and thermal stability also make them a promising alternative to traditional antimicrobials (Wayah *et al.*, 2024).

Weissella spp. is one of the groups of bacteriocin-producing lactic acid bacteria. Consequently, a number of different strains of *Weissella* spp. are garnering a lot of attention as possible probiotics (Kang *et al.*, 2023). Even though a large number of strains have been extensively characterized, only a small number of bacteriocins derived from *Weissella* species have been found. Bacteriocins from *Weissella* spp. include weissellin A (Papagianni and Papamichael, 2011; Papagianni and Sergelidis, 2013), weissellicin 110 (Srionnual *et al.*, 2007), bacteriocins 7293A and 7293B (Woraprayote *et al.*, 2015), weissellicin D (Chen, Chen, *et al.*, 2014; Chen, Rui, *et al.*, 2014), weissellicin Y and M (Masuda *et al.*, 2012, 2016), weissellicin MBF, Bac1, Bac2, and Bac3 (Malik *et al.*, 2020).

The stability of bacteriocins with respect to temperature, chemical, and pH is an important biophysical property that determines their potential for usage in industrial and medicinal applications. Heat stability of Weissellicin L was established by its ability to have residual activity even after being heated to 121°C for 15 minutes (Leong *et al.*, 2013). Stability of Weissellicin D activity was shown throughout a broad pH range spanning from 2 to 8. In another report, weissellicin D exhibited excellent thermal stability such that its activity remained unaffected by incubation at all temperatures examined (-20^{0}C to 121^{0}C). The bacteriocin displayed sustained activity even after undergoing autoclaving at 121°C for 15 minutes. Neither organic reagents, methanol and ethanol, nor surfactants, Tween 80, sodium dodecyl sulfate (SDS), and urea, affected the antibacterial activity of Weissellicin D (Chen, Chen, *et al.*, 2014). Despite the potentials of bacteriocins from *Weissella cibaria*, their biophysical characteristics remain inadequately investigated. In Nigeria, there is no published information on biophysical attributes of bacteriocins from indigenous *Weissella cibaria*. Therefore, this study was carried out to investigate the biophysical characteristics of a bacteriocin from *Weissella cibaria* man1.

MATERIALS AND METHODS

Collection of sample

Bacteriocin-producing *Weissella cibaria* man1 was obtained from the culture collection of Department of Biotechnology, Kaduna State University. *Weissella cibaria* man1 was maintained on De man Rogosa Sharpe (MRS) agar (Merck, Darmstadt, Germany). *Rhizopus stolonifer* was obtained from the Department of Microbiology, Kaduna State University and maintained on sabouraud dextrose agar (Merck, Darmstadt, Germany).

Procedures on Biophysical characterization of bacteriocin from *Weissella cibaria* man1

Thermal stability test

Thermal, pH and chemical stability of bacteriocin from *Weissella cibaria* man1 was determined as described by Wayah and Philip (Samson Baranzan Wayah and Philip, 2018). *Weissella cibaria* man1 was inoculated into sterile MRS broth and incubated for 24 hours at 37^{0}C. The culture was centrifuged (10,000×g for 20 min at 4°C) to obtain the supernatant which was filtered

using 0.22 µm filter to form cell-free supernatant (CFS) referred to as the crude bacteriocin. Thermal stability of the bacteriocin was determined by heating it at various temperatures (40°C, 60°C, 80°C, 100°C) for 30 minutes, followed cooling to room temperature and then, measuring residual antimicrobial activity by agar well diffusion assay (AWDA) using *Rhizopus stolonifer* as the indicator of antimicrobial activity. Effect of heating duration on antimicrobial activity of the bacteriocin was investigated by heating the CFS at 80^0C and 100^0C for durations of 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, and 51 minutes at both temperatures. After cooling of the heated CFS to room temperature, AWDA assay was performed using *Rhizopus stolonifer* as the indicator of antimicrobial activity. The agar well diffusion assay utilized MRS agar enriched with 0.1% $CaCO_3$ (Friedemann Schmidt Chemical, Germany) to neutralize the acidity. For all stability tests, the control was the untreated CFS.

The pH and chemical stability test

In the case of pH stability test, the CFS was adjusted to various pH values (2, 4, 6, 8) and incubated for 2 hours at room temperature, thereafter, antimicrobial activity was measured using AWDA while employing *Rhizopus stolonifer* as the indicator of inhibitory activity. Chemical stability test was performed by exposing the CFS to Tween 80 and sodium dodecyl sulfate (SDS) ensuring a final concentration of 1% (v/v) for each of the chemical. The mixture was incubated for 2 hours at room temperature. Afterwards, antimicrobial activity was measured by AWDA using *Rhizopus stolonifera* as the indicator (Samson B. Wayah and Philip, 2018). For all stability tests, the control was the untreated CFS. The agar well diffusion assay employed MRS agar supplemented with 0.1% $CaCO_3$ (Friedemann Schmidt Chemical, Germany) to neutralize the acidity.

Data analysis

Experiments were done in triplicates from which mean values and standard deviations were calculated. Inferential statistics involved one-way analysis of variance using IBM SPSS statistics software version 29. Mean values that are significantly different at 95% confidence level were assigned different letters. The highest mean value was assigned letter "a".

RESULTS AND DISCUSSION

Thermal stability

Temperature had a significant effect ($p < 0.05$) on antimicrobial activity of bacteriocin from *Weissella cibaria* man1. It was observed that the higher the temperature the lower the antimicrobial activity of the bacteriocin (Table 1). Heating at 40^0C for 10 minutes had no significant impact on the inhibitory activity of the bacteriocin. But beyond 40^0C, significant decline in antimicrobial activity was observed. Inhibitory activity of the bacteriocin was highest when treated at 100^0C with zone of inhibition of 13.5 ± 0.13 mm (Table 1). The presence of residual antimicrobial activity even at 100^0C is indicative of its thermal stability. A study revealed that weissellicin 110 from *Weissella cibaria* 110 was heat-stable due to the occurrence of residual inhibitory activity even after heating at 121°C for 15 min (Srionnual *et al.*, 2007). Bacteriocin N23 from *Weissella cibaria* N23 had residual inhibitory activity after heating at 60-100^0C for 20 minutes and 121^0C for 15 minutes (Pringsulaka *et al.*, 2012). Another investigation revealed that bacteriocin from *Weissella confusa* A3 retained inhibitory activity after heating at 100°C for 30 minutes, indicating its thermal stability (Goh and Philip, 2015). Weissellicin L isolated from *Weissella hellenica* 4-7 is heat-stable up to a temperature of 121^0C (Leong *et al.*, 2013). Weissellicins Y and M from *Weissella hellenica* QU 13 are both thermostable. However, Weissellicin M is more heat-stable than Weissellicin Y (Masuda *et al.*, 2012).

Furthermore, temperature was observed to decrease inhibitory activity of the bacteriocin in a time dependent manner (Table 2). The least effect was seen after 10 minutes (15.9 ± 0.11 mm when heated at 80^0C and 13.7 ± 0.08 mm when heated at 100^0C) while the most was observed after 51 minutes (9.6 ± 0.21 mm when heated at 80^0C and 5.2 ± 0.05 mm when heated at 100^0C) (Table 2).

Table 1: Effect of temperature on antimicrobial activity of bacteriocin from *Weissella cibaria* man1.

Temperature (^{0}C)	Zone of inhibition (mm)
40	18.3 ± 0.13^a
60	17.0 ± 0.24^b
80	15.6 ± 0.08^c
100	13.5 ± 0.11^d
Control	18.5 ± 0.32^a

Values are means of triplicate measurements ± standard deviation. Mean values that differ significantly at 95% confidence level were assigned different letters.

Table 2: Effect of heating duration on antimicrobial activity of bacteriocin from *Weissella cibaria* man1.

Heating duration (minutes)	Zone of inhibition (mm)	
	Heating at 80^0C	Heating at 100^0C
10	15.9 ± 0.11^b	13.7 ± 0.08^b
15	15.8 ± 0.09^b	12.1 ± 0.16^c
20	14.2 ± 0.14^c	11.1 ± 0.25^d
25	13.0 ± 0.23^d	9.8 ± 0.31^e
30	12.3 ± 0.06^d	7.7 ± 0.27^f
51	9.6 ± 0.21^e	5.2 ± 0.05^g
Control	18.5 ± 0.32^a	18.5 ± 0.32^a

Values are means of triplicate measurements ± standard deviation. Mean values that differ significantly at 95% confidence level were assigned different letters.

The pH and chemical stability

The pH significantly affected antimicrobial activity of bacteriocin from *Weissella cibaria* man1 at 95% confidence level. The bacteriocin had significantly higher inhibitory activity at low pH values than at high pH values (Table 3). A study observed that pH had a significant effect on antimicrobial activity of a bacteriocin from *Weissella cibaria* FMF4B16. Additionally, these authors revealed that the bacteriocin had higher inhibitory activity at low pH values (4, 5) but significantly lower antimicrobial activity at pH value of 6 (Ndagano *et al.*, 2011). In another investigation, involving bacteriocin N23 from *Weissella cibaria* N23, it was observed that bacteriocin N23 had inhibitory activity at pH values between 2 to 8 but became inactive at pH value of 10 (Pringsulaka *et al.*, 2012). Bacteriocin from *Weissella confusa* A3 had higher antimicrobial activity at pH value of 2 to 6 (Goh and Philip, 2015). Weissellicin M from *Weissella hellenica* QU 13 displayed high antimicrobial activity at a pH value of 3 (Leong *et al.*, 2013).

Table 3: Effect of pH on antimicrobial activity of bacteriocin from *Weissella cibaria* man1.

pH	Zone of inhibition (mm)
2	20.5 ± 0.04^c
4	22.3 ± 0.23^b
6	14.1 ± 0.09^d
8	10.5 ± 0.15^e
Control	18.2 ± 0.22^a

Values are means of triplicate measurements ± standard deviation. Mean values that differ significantly at 95% confidence level were assigned different letters.

Treating bacteriocin from *Weissella cibaria* man1 with Tween 80 and SDS had no significant effect on its antimicrobial activity (Table 4). A study showed that enterocin NKR-5-3B retained all its antimicrobial activity after treatment with 6 M urea (Wang *et al.*, 2023). In another research, salivaricin 9 from *Streptococcus salivarius* NU10 was stable to treatment with Tween 20, Tween 80, Triton X-100, SDS, ethylenediamine tetraacetic acid (EDTA), urea and NaCl (Barbour *et al.*, 2013). Treatment with surfactants (Tween 20, Tween 80, EDTA, Triton X-100) had no significant effect on antimicrobial activity of plantaricin K25 from *Lactobacillus plantarum* (Wen *et al.*, 2016).

Table 4: Effect of chemicals on antimicrobial activity of bacteriocin from *Weissella cibaria* man1.

Chemical	Zone of inhibition (mm)
Tween 80	17.9 ± 0.19^{a}
SDS	17.7 ± 0.21^{a}
Control	18.4 ± 0.14^{a}

Values are means of triplicate measurements ± standard deviation. Mean values that differ significantly at 95% confidence level were assigned different letters.

CONCLUSION

Temperature had a significant effect on antimicrobial activity of bacteriocin from *Weissella cibaria* man1 in a time dependent manner. However, the bacteriocin had residual antimicrobial activity up to a temperature of 100^{0}C, indicating its thermal stability. The bacteriocin had higher inhibitory activity at low pH values (2 and 4) than at higher pH values (6 and 8). Further, antimicrobial activity of bacteriocin from *Weissella cibaria* man1 was not affected by treatment with Tween 80 and SDS, indicating its chemical stability. The medical and industrial applications of this bacteriocin will be further facilitated as a result of these discoveries.

REFERENCES

Barbour, A., Philip, K., and Muniandy, S. (2013) Enhanced Production, Purification, Characterization and Mechanism of Action of Salivaricin 9 Lantibiotic Produced by *Streptococcus salivarius* NU10. *PLoS One* **8**:.

Chen, C., Chen, X., Jiang, M., Rui, X., Li, W., and Dong, M. (2014) A newly discovered bacteriocin from *Weissella hellenica* D1501 associated with Chinese Dong fermented meat (Nanx Wudl). *Food Control* **42**: 116–124.

Chen, C., Rui, X., Lu, Z., Li, W., and Dong, M. (2014) Enhanced shelf-life of tofu by using bacteriocinogenic *Weissella hellenica* D1501 as bioprotective cultures. *Food Control* **46**: 203–209.

Cheruvari, A. and Kammara, R. (2024) Bacteriocins Future Perspectives: Substitutes to Antibiotics. *Food Control* 110834.

Darby, E.M., Trampari, E., Siasat, P., Gaya, M.S., Alav, I., Webber, M.A., and Blair, J.M.A. (2023) Molecular mechanisms of antibiotic resistance revisited. *Nat Rev Microbiol* **21**: 280–295.

Denku, C.Y., Ambelu, A., and Mitike, G. (2022) Enteric bacterial pathogens and their antibiotic-resistant patterns from the environmental sources in different regions of Ethiopia: A laboratory-based cross-sectional study. *Health Sci Rep* **5**: e521.

Goh, H.F. and Philip, K. (2015) Purification and Characterization of Bacteriocin Produced by *Weissella confusa* A3 of Dairy Origin. *PLoS One* **10**: e0140434.

Kang, C.E., Park, Y.J., Kim, J.H., Lee, N.K., and Paik, H.D. (2023) Probiotic *Weissella cibaria* displays antibacterial and anti-biofilm effect against cavity-causing Streptococcus mutans. *Microb Pathog* **180**: 106151.

Leong, K.H., Chen, Y.S., Lin, Y.H., Pan, S.F., Yu, B., Wu, H.C., and Yanagida, F. (2013) Weissellicin L, a novel bacteriocin from sian-sianzih-isolated *Weissella hellenica* 4-7. *J Appl Microbiol* **115**: 70–76.

Malik, A., Yuliantie, E., Suprahman, N.Y., Linardi, T., Widiyanti, A.W., Haldy, J., et al. (2020) Construction and Functional Analysis of the Recombinant Bacteriocins Weissellicin-MBF from *Weissella confusa* MBF8-1. *Curr Pharm Biotechnol* **22**: 115–122.

Masuda, Y., Perez, R.H., Zendo, T., and Sonomoto, K. (2016) Nutrition-adaptive control of multiple-bacteriocin production by *Weissella hellenica* QU 13. *J Appl Microbiol* **120**: 70–79.

Masuda, Y., Zendo, T., Sawa, N., Perez, R.H., Nakayama, J., and Sonomoto, K. (2012) Characterization and identification of weissellicin Y and weissellicin M, novel bacteriocins produced by *Weissella hellenica* QU 13. *J Appl Microbiol* **112**: 99–108.

Ndagano, D., Lamoureux, T., Dortu, C., Vandermoten, S., and Thonart, P. (2011) Antifungal Activity of 2 Lactic Acid Bacteria of the Weissella Genus Isolated from Food. *J Food Sci* **76**: M305–M311.

Papagianni, M. and Papamichael, E.M. (2011) Purification, amino acid sequence and characterization of the class IIa bacteriocin weissellin A, produced by *Weissella paramesenteroides* DX. *Bioresour Technol* **102**: 6730–6734.

Papagianni, M. and Sergelidis, D. (2013) Effects of the presence of the curing agent sodium nitrite, used in the production of fermented sausages, on bacteriocin production by *Weissella paramesenteroides* DX grown in meat simulation medium. *Enzyme Microb Technol* **53**: 1–5.

Pepi, M., Barretta, R., Barache, N., Belguesmia, Y., Martinez, B., Seal, B.S., and Drider, D. (2024) Bacteriocins and Bacteriophages as Dual Biological Players for Food Safety Applications. *Encyclopedia 2024, Vol 4, Pages 79-90* **4**: 79–90.

Pringsulaka, O., Thongngam, N., Suwannasai, N., Atthakor, W., Pothivejkul, K., and Rangsiruji, A. (2012) Partial characterisation of bacteriocins produced by lactic acid bacteria isolated from Thai fermented meat and fish products. *Food Control* **23**: 547–551.

Srionnual, S., Yanagida, F., Lin, L.H., Hsiao, K.N., and Chen, Y.S. (2007) Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110, isolated from plaa-som, a fermented fish product from Thailand. *Appl Environ Microbiol* **73**: 2247–2250.

Wang, C.K., Huang, Y.H., Shabbir, F., Pham, H.T., Lawrence, N., Benfield, A.H., et al. (2023) The circular bacteriocin enterocin NKR-5-3B has an improved stability profile over nisin. *Peptides (NY)* **167**:.

Wayah, Samson Baranzan and Philip, K. (2018) Characterization, yield optimization, scale up and biopreservative potential of fermencin SA715, a novel bacteriocin from *Lactobacillus fermentum* GA715 of goat milk origin. *Microb Cell Fact* **17**: 125.

Wayah, Samson B. and Philip, K. (2018) Pentocin MQ1: A novel, broad-spectrum, pore-forming bacteriocin from *Lactobacillus pentosus* CS2 with quorum sensing regulatory mechanism and biopreservative potential. *Front Microbiol* **9**: 338656.

Wayah, S.B., Tanko, C., Faila, A.A., Yahaya, G., and Aji, R.J. (2024) Unveiling the Genetic Basis of Bacteriocin Production from *Enterococcus faecium* ATCC 19434. *FUDMA Journal of Sciences* **8**: 297–301.

Wen, L.S., Philip, K., and Ajam, N. (2016) Purification, characterization and mode of action of plantaricin K25 produced by *Lactobacillus plantarum*. *Food Control* **60**: 430–439.

Woraprayote, W., Pumpuang, L., Tosukhowong, A., Roytrakul, S., Perez, R.H., Zendo, T., et al. (2015) Two putatively novel bacteriocins active against Gram-negative food borne pathogens produced by *Weissella hellenica* BCC 7293. *Food Control* **55**: 176–184.