



PURIFICATION AND PARTIAL CHARACTERISATION OF AN ANTIFUNGAL BACTERIOCIN FROM *Bacillus* sp. Sh10 ASSOCIATED WITH MARINE CARPET CLAM (*Paphia textile*)

Fatemeh Shayesteh^{*1}, Asmat Ahmad² & Gires Usup³

Address(es):

¹Department of Fisheries, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas, Iran.

²School of Bioscience and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

³School of Environmental Science and Natural Resources, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

*Corresponding author: Shayesteh_fatemeh@yahoo.com

<https://doi.org/10.15414/jmbfs.2513>

ARTICLE INFO

Received 4. 1. 2020
Revised 13. 1. 2021
Accepted 20. 1. 2021
Published 1. 6. 2021

Regular article



ABSTRACT

This study was conducted to purify and characterize an anti- *C. albicans* bacteriocin produced by *Bacillus* sp. Sh10 isolated from marine carpet clam, *Paphia textile*. Extracellularly produced bacteriocin was purified by 80 % ammonium sulfate precipitation and size exclusive gel chromatography using superdex-200 column chromatography. The purification steps resulted in 3.68-fold increase in specific activity and recovery of 20.66 % of total activity. The purity rate and molecular mass of 11 kDa of this compound were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Profile analysis of this bacteriocin by electrospray mass spectrometry (LC/MS/MS) showed 43% protein sequence coverage with a putative uncharacterized protein from *Bacillus cereus* strain B4264.

Keywords: Marine bacteria, antifungal bacteriocin, *Candida albicans*, *Bacillus* sp., purification

INTRODUCTION

Bacteriocins are generally defined as antimicrobial peptides produced by bacteria that inhibit or kill microorganisms closely-related or non-related to produced bacteria (Leroy & De Vuyst, 2004; Cotter *et al.*, 2005).

The members of *Bacillus* genus are known to produce a variety of bacteriocins consisting of a hydrophobic fatty acid linked to a hydrophilic peptide chain (Roongsawang *et al.*, 2011). This complex structural diversity warrants the broad spectra of inhibition of some *Bacillus* strains, which includes not only Gram-positive and Gram-negative bacteria, but fungi and amoeba as well (Benitez *et al.*, 2011; Shelburne *et al.* 2007; Cladera-Olivera *et al.*, 2004).

The pathogenicity of *C. albicans* makes it particularly troublesome for patients with immunosuppression conditions. Indeed increasing numbers of diabetics, HIV type-C patients, post-surgical patients, premature infants, and trauma patients are progressively vulnerable to *C. albicans* infections. These infections can be localized to the gastrointestinal, oral, or vaginal tracts, or be disseminated across multiple sites and organs, which can be fatal. The mortality rate in patients infected with *C. albicans*, exceeds 40% (Gudlaugsson *et al.*, 2003).

During the past five decades, systemic antifungal treatments have been devised (Ostrosky-Zeichner *et al.*, 2010); yet, new antifungals are urgently needed to offset the rising incidence of invasive fungal infections arising from *C. albicans* and other pathogens becoming drug-resistant (Shahin *et al.*, 2015; Amel *et al.*, 2015; Adebayo & Aderiyi, 2011). To be effective, these new treatments will need to go beyond conventional drug targets, to focus instead upon new targets such as novel metabolic pathways or virulence factors.

This report describes the partial purification and identification of anti-*C. albicans* bacteriocin, which is produced by *Bacillus* sp. sh10 isolated from *Paphia textile*.

MATERIAL AND METHODS

Isolation, detection and partial purification of bacteriocin

In the present study, a bacteriocin produced from *Bacillus* sp. sh10 was used. This microorganism was isolated previously from carpet clam (*P. textile*) (Shayesteh *et al.*, 2014, 2015). Inorganic salt medium (Pridham & Gottlieb, 1948) containing 2% tryptone and 1% glucose as nitrogen and carbon source, respectively followed by 2% sodium chloride was obtained as an optimized medium for bacteriocin production by isolate sh10 (Shayesteh *et al.*, 2014). In

addition, the optimized culture media was adjusted to pH 8.0 and bacteria culture was incubated at 30 °C under the aerobic condition for 30 h. Afterwards, the supernatant was achieved by centrifuging bacteria culture at 3000 × g for 30 min at 4 °C. The supernatant which was obtained after centrifugation was then filtered by 0.22 µm membrane filter followed by ammonium sulfate precipitation at a different percentage of saturation (40 % - 60 % and 80 %) at 4 °C. After moderate agitation, the precipitated protein obtained by centrifugation (3000 g, 30 min, 4 °C), was dissolved in 0.1 M phosphate buffer (pH 7.0) followed by dialysing via dialysis membrane (2 kDa cut-off, sigma) against the same buffer at 4 °C for 24 h (Rajaram *et al.*, 2010) and applied as ammonium sulfate fraction.

The antimicrobial activity of bacteriocin against *C. albicans* ATCC 10231 was determined using the spot-on-lawn method (Seuk-Hyun & Cheol, 2000). In brief, in each step of purification, 10 µL of bacteriocin was spotted onto the surface of tryptone soy agar overlaid by 1.5 × 10⁸ CFU/mL cells of the *C. albicans* followed by incubation at 30 °C for 48 h. A bacteriocin titer was determined as the reciprocal of the highest twofold dilution indicating the complete inhibition of the indicator lawn and was presented in activity units (AU) per ml of culture media. The titer of the bacteriocin solution, in AU/mL, was calculated as (1000/d) D, where D is the dilution factor and d is the dose (Hoover & Harlander, 1993).

Gel filtration chromatography

The dialyzed protein was placed in a Superdex-200 column (AKTA purifier UPC-900) at room temperature. The protein was eluted with phosphate buffer (0.1 M, pH 7.0) at a flow rate of 0.5 mL/min and screened at 280 nm. Fractions (1 mL) were collected and evaluated in terms of antimicrobial activity by spotting aliquots (10 µL) on a lawn of *C. albicans*, as described previously. The molecular weight of bacteriocin was calculated through gel filtration with aprotinin (6.5 kDa), ribonuclease A (13.7 kDa), carbonic anhydrase (29 kDa), ovalbumin (44 kDa), conalbumin (75 kDa), aldolase (158 kDa), and ferritin (440 kDa) as references.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

The active fractions acquired from gel filtration chromatography were analyzed by 15% SDS-PAGE and Tris-Glycine buffer system (Laemmli, 1970) to verify the purity. A pre-stained broad-range molecular mass marker (11- 245 KDa, GeneDirex) was utilized to assess the bacteriocin molecular weight. In order to enter the samples into the separating gel, the electrophoresis was performed at 50 V and was followed by a constant power of 100 V until samples achieved the endpoint of the separating gel. Fractions containing a single band were pooled and designed as gel filtrate fractions.

Direct detection of the bacteriocin activity on SDS-PAGE

The protein band activity in SDS-PAGE was assayed as described by Cherif et al. (2001). After the samples were run, the gel was vertically cut into two halves. The first half was stained by Coomassie blue R-250 to observe the protein bands; the other half was rinsed with deionized water for 2 h with intermediate steps of changing the water every 20 min. Afterward, the gel was placed in a sterile Petri dish and subjected to UV for 20 min for sterilization. The gel was then overlaid with Mueller Hinton soft agar seeded with 1.5×10^8 CFU/mL cells of *C. albicans* followed by incubation at 30 °C. After 24-48 h of incubation, the gel was examined to determine the growth inhibition zone caused by bacteriocin.

Determination of protein concentration

The protein concentrations of the samples obtained from culture supernatant, ammonium sulfate precipitation, and gel filtration were determined in accordance with Bradford assay (Bradford, 1976). Bradford solution was added to 100 µL aliquots containing different bovine serum albumin (BSA) concentrations and bacteriocin samples. These samples were incubated at room temperature for 10 min. The absorbance at 595 nm was also determined using a spectrophotometer. BSA standards were used to create a standard curve, and the protein concentration of the samples was calculated on the basis of this standard curve.

Identification of bacteriocin using mass spectrometry (LCMS/MS)

Active bands on SDS-PAGE corresponding to a molecular weight of approximately 11 kDa was excised from the gel. The process involved in-gel destaining, reduction with dithiothreitol, alkylation of the thiols with iodoacetamide, and in-gel tryptic digestion and extraction of the resulting peptides (Proteomics International Facility, Australia). The extracted peptides were analyzed through electrospray ionization mass spectrometry by Agilent 1260 Infinity HPLC System coupled with an Agilent 6540 mass spectrometer. Tryptic peptides were loaded onto a 5 µm 300 SB C18 column (Agilent) and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v). The spectra were analyzed to recognize the target proteins by Mascot sequence matching software [Matrix Science] with Ludwig NR database.

RESULTS

Extraction, purification, and molecular weight determination of bacteriocin produced by *Bacillus* sp. Sh10

The highest bacteriocin activity was found at 80% of ammonium sulfate precipitation, which resulted in a 3.68-fold increase in the specific activity. This purification step resulted in 494.42 AU/mg and 20.66 % of specific activity and total activity recovery, respectively (Table 1). The separation profile of gel filtration chromatography (superdex-200) achieved from crude ammonium sulfate precipitate, resulted in two major peaks (Figure 1). Among 36 fractions collected, antimicrobial activity was trailed in fractions 23-27 which were located in the second peak (Figure 1). When active fractions obtained from gel chromatography were compared to the standard curve, the molecular weight of eluted peptides in these fractions corresponded to ~13-6 kDa. The present purification step resulted in 546.22 AU/mg and 6.5% of specific activity and total activity recovery, respectively (Table 1). The ammonium sulfate precipitate and active fractions of bacteriocin collected from size exclusion gel chromatography were examined by SDS-PAGE (Figure 2). The accurate molecular weight of antimicrobial peptide was measured by SDS-PAGE. In the case of ammonium sulfate precipitation, seven major and other bands were observed with different molecular weights compared with a broad range molecular marker (Figure 2A). The bacteriocin activity against *C. albicans* ATCC 10231 corresponded to a low-molecular-weight band with an estimated molecular mass of 11 kDa (Figure 2B). Based on the active fractions from gel chromatography, fractions 23 and 24 contained more than one band, indicating the impurity of these fractions. However, fractions 25, 26, and 27 showed single sharp bands as indicated by SDS-PAGE (Figure 2). The bacteriocin activity of fractions 23–27 on SDS-PAGE against *C. albicans* ATCC 10231 (Figure 2B) was exhibited as a clear zone of inhibition on the corresponding band (~11 kDa). Therefore, fractions 25, 26, and 27 with the single band were pooled and designed as gel filtrate fraction of bacteriocin.

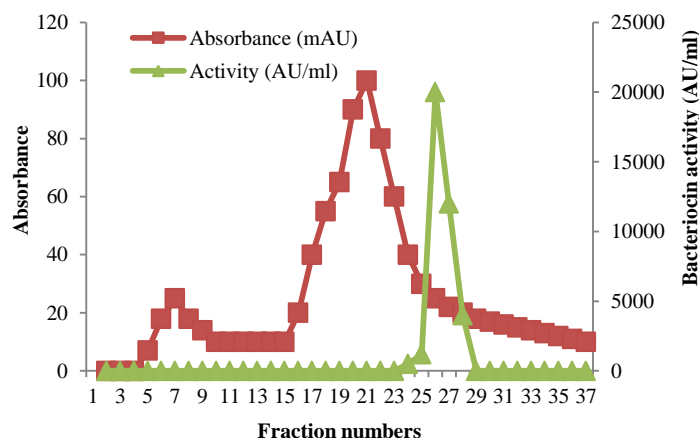


Figure 1 Elution profile of bacteriocin from isolate Sh10 on superdex-200 column chromatography

Table 1 Purification steps of bacteriocin produced by isolate Sh10

Purification steps	Volume (mL)	Activity unit ¹ (AU/mL)	Protein ² (mg/mL)	Total protein ³ (mg)	Total activity ⁴ (AU)	Specific activity ⁵ (AU/mg)	Purification fold ⁶	Recovery ⁷ (%)
Culture supernatant	1000	1200	8.93	8930	1200000	134.38	1	100
Ammonium sulfate precipitation (80%)	80	3100	6.27	501.6	248000	494.42	3.68	20.66
Gel filtration	30	2600	4.76	142.8	78000	546.22	4.06	6.5

¹Activity unit (AU/mL) = Reciprocal of the highest dilution × 1000/volume of bacteriocins added; ²Protein concentration measured by Bradford's method; ³Total protein = protein concentration × total volume; ⁴Total activity = Activity (AU/mL) × Total volume; ⁵Specific activity (AU/mg) = Total activity of the subsequent purification step/Total protein of the same step; ⁶Fold purification = Specific activity of subsequent step/ Specific activity of crude preparation; ⁷Recovery (%) = Total activity of subsequent step × 100/Total activity of crude preparation.

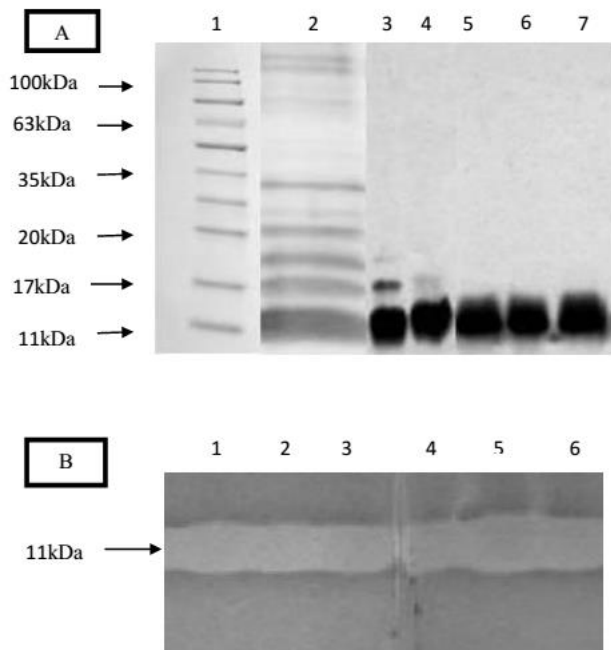


Figure 2 SDS-PAGE profile of bacteriocin produced by *Bacillus* sp. Sh10. A, Gel stained with coomassie blue R250. Lane 1, broad range molecular weight marker (Gen Direx); lane 2, bacteriocin precipitated with 80% ammonium sulfate; lanes 3,4,5,6,7, gel exclusive chromatography fractions 23-27 respectively; B, SDS-Polyacrylamide gel overlaid with *C. albicans* ATCC 10231 showing the inhibitory effect (arrow) of bacteriocin. Lane 1, bacteriocin precipitated with 80% ammonium sulfate; lanes 2,3,4,5,6, gel exclusive chromatography fractions 23-27, respectively.

Identification of bacteriocin by LC-MS/MS

The gel-filtration purified band that showed anti-*Candida* activity in SDS-PAGE gel overlay assay was exposed to LC-MS/MS analysis. The result from Mascot Sequence Matching Software [Matrix Science] with Ludwig NR database showed 43% identity of bacteriocin produced by isolate Sh10 with uncharacterized protein Tax_Id=405532 from *B. cereus* [strain B4264]. Out of 105 amino acids of the uncharacterized protein Tax_Id=405532, 46 amino acids were detected by LC-MS/MS, and the subsequent data analysis showed a 43% sequence similarity with the score of 175 with the uncharacterized protein Tax_Id=405532 from *B. cereus* [strain B4264] (Figures 3 and 4). Based on the LC-MS/MS, the peaks corresponding to NFNTIDVTMNLNELYVLSGR (score: 66, expected value: 0.0085), SIDILSGDK (score: 18, expected value: 1.3e+03), SIDILSGDK (score: 33, expected value: 41), SIDILSGDKKEAIQLNK (score: 70, expected value: 0.0043), and EAIQLNKYTISFNKPGK (score: 5, expected value: 1.3e+04) were detected in the mass spectra (Figure 3A-E). In addition, the detected amino acids by LC-MS/MS did not show identity or homology to the antimicrobial peptides available at the antimicrobial peptide database. The overall view of the matched protein is summarized below.

Protein view:
 MASCOT search result: Protein tr|B7HDM0|BCB4264_A3844 B7HDM0_BACC4 Uncharacterized protein n=3 Tax_Id=405532 [*Bacillus cereus* (strain B4264)]
 Database: Ludwig NR
 Score: 175
 Nominal mass (M_r): 11558 Da
 Calculated PI: 7.77
 Protein sequence coverage: 43%

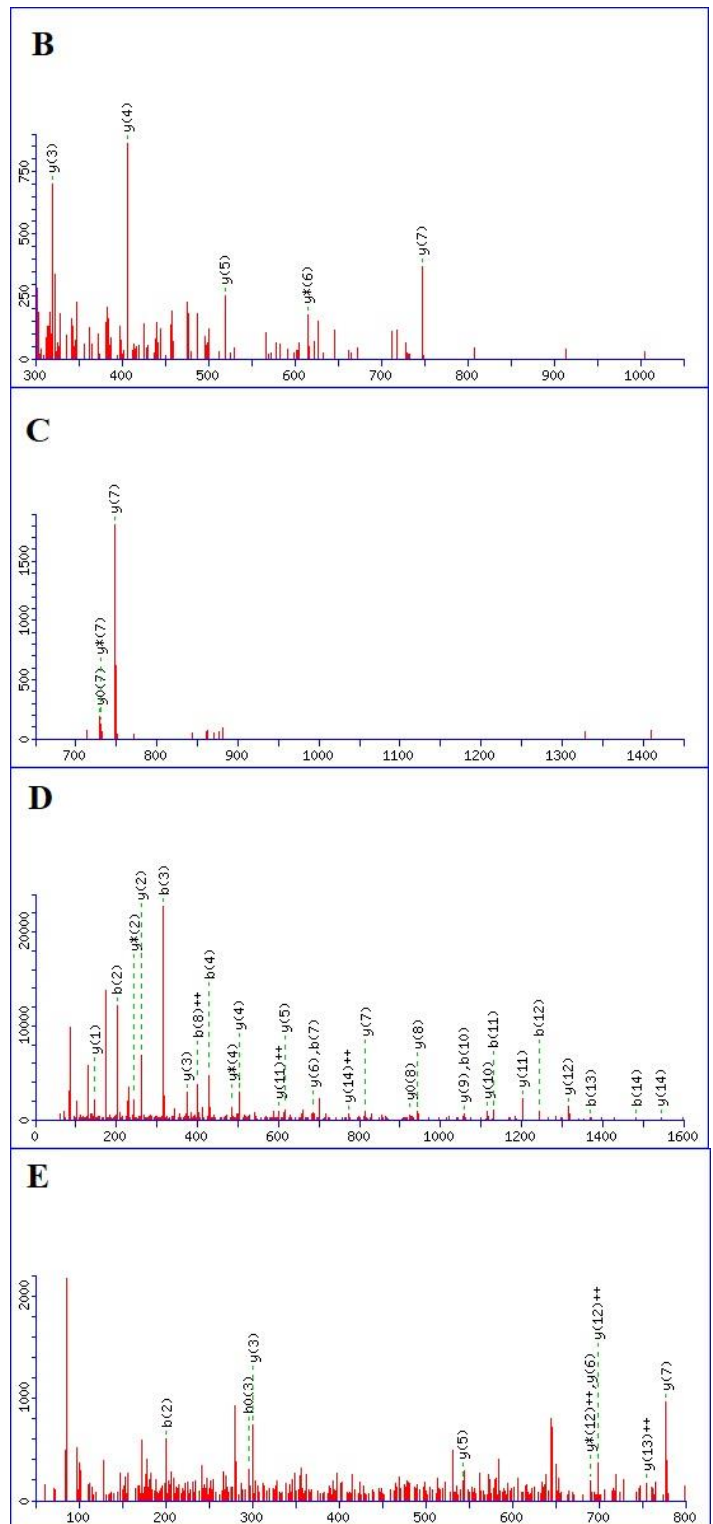
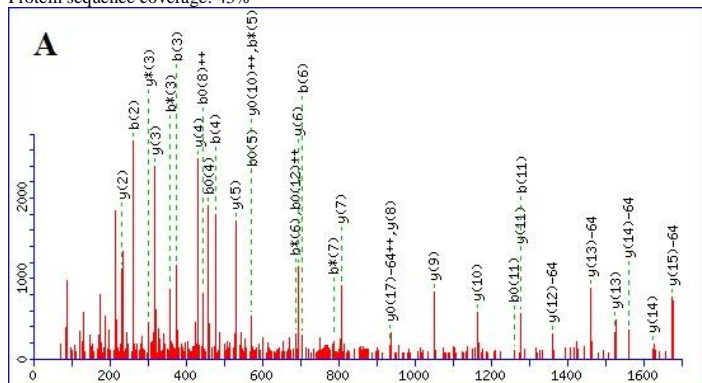


Figure 3 MALDI-TOF spectra of peptide; (A) peptide NFNTIDVTMNLNELYVLSGR, (B) peptide SIDILSGDK, (C) peptide SIDILSGDK, (D) peptide SIDILSGDKKEAIQLNK and (E) peptide EAIQLNKYTISFNKPGK.

1 MKQIKKFAV ALAGTIGLSG LLFLEPSVSV AEAQQVKVKN FNTIDVTMNL
 51 NELYVLSGRS IDILSGDKEA IQLNKYTISF NKPGKYVIKV NGCIYDDVYT
 101 FIVNE

Figure 4 Amino acid sequence of uncharacterized protein n=3 Tax_Id=405532 [*Bacillus cereus* (strain B4264)]. The underline sequences reveal those that matched with that of experimental sample from SDS-PAGE active band. The sequence coverage was 43 % with a score of 175 [Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits. Ions score is -10*Log(P), where P is the probability that the observed match is a random event. Individual ions scores > 59 indicate identity or extensive homology (p<0.05)].

DISCUSSION

Protein fractionation is usually performed through ammonium sulfate precipitation as it facilitates concentration of dilute protein solutions (Whitford et al., 2001). This approach also prevents protein denaturation and makes it possible to restore activity after re-dissociation. Proteolysis and contamination by bacteria of proteins are hindered by ammonium sulfate as well (Harris & Angal, 1989). Enhanced purification is generally achieved by taking an ammonium sulfate cut. Bioactivity assays of the ammonium sulfate precipitate fraction and active fractions from gel filtration chromatography on SDS-PAGE suggested that inhibitory activity was due to a low molecular weight protein (~11 kDa). Bacteriocin activity can be most easily, accurately, and rapidly identified through the common method of SDS-PAGE (Crupper & Iandolo, 1996).

In our previous study, we demonstrated that among different enzymes tested for bacteriocin sensitivity, lysozyme, α -amylase, lipase, and proteinase K did not alter bacteriocin activity, but complete inactivation was observed after treatment with trypsin. The inhibitory compound was heat resistant and the antimicrobial activity of the present bacteriocin did not alter after autoclaving (Shayesteh et al., 2015). Although the present bacteriocin possessed the same range of molecular weight as other bacteriocins from *B. cereus* group members but differed in physico-chemical characteristics. For example, cerein GN105 (9 kDa) produced by *B. cereus* GN105 (Naclerio et al., 1993) differed from the present bacteriocin in terms of heat stability (stable until 75 °C). Tochicin (10.5 kDa) and thuricin (11.6 kDa) produced by *B. thuringiensis* ssp. *tochigiensis* HD868 (Paik et al. 1997) and *B. thuringiensis* BMG1.7 (Cherif et al., 2001), respectively, both differed from the present bacteriocin in terms of proteinase K susceptibility and heat stability (complete inactivation by heating at 121 °C for 20 min). However, SDS-PAGE identifies as BLIS of approximately 10 kDa compounds synthesised by Mexican *B. thuringiensis*, including Morricon 269 from *B. thuringiensis* ssp. *morrisoni* (LBIT 269), kurstacin 287 from *B. thuringiensis* ssp. *kurstaki* (LBIT 287), kenyacin 404 from *B. thuringiensis* ssp. *kenyae* (LBIT 404), entomocin 420 from *B. thuringiensis* ssp. *entomocidus* (LBIT 420), and tolworthin 524 from *B. thuringiensis* ssp. *tolworthi* (LBIT 524) (Barboza-Corona et al., 2007; De la Fuente-Salcido et al., 2008). Depending on heat stability, two uniform groups of such BLIS was distinguished via partial purification. The first group comprises morricon 269 and kurstacin 287, which show tolerance to heat and retain their stability up to 80°C. Meanwhile, the second group consists of the rest of the BLIS, which exhibit resistance to heat and retain their stability up to 121°C. The characteristic which differentiates BLIS from second group from the present bacteriocin is their complete loss of activity after exposure to proteinase K. The heat resistance, protease sensitivity, and low molecular weight of the present bacteriocin indicate that it belongs to group II bacteriocin (Klaenhammer, 1993).

The gel-purified band corresponding to approximately 11 kDa was analyzed by LC-MS/MS. Mascot software showed 43% similarity to an uncharacterized protein from *B. cereus* B4264 (46 out of 105 amino acids showed homology). We compared the present bacteriocin's amino acid sequence with those of other bacteriocins in the NCBI and Antimicrobial Peptide Database. Sequence comparison showed no matches to the partial sequence obtained from LC-MS/MS and to the uncharacterized protein from *B. cereus* B4264. The non-homology between the present bacteriocin and previously isolated bacteriocins confirms that the bacteriocin produced by isolate Sh10 is a novel bacteriocin.

The present bacteriocin has a high amount of cationic amino acids, a net charge of +1, and a high percentage of hydrophobicity (42%). Given these characteristics, bacteriocin produced by *Bacillus* sp. Sh10 may exert its action by interacting with cell-wall-associated or membrane-associated binding sites in sensitive *C. albicans* cells. These findings indicated that the net positive charge of present bacteriocin would interact with the negative charges of phosphate groups in the cell walls of sensitive cells by a strong electrostatic interaction, resulting in pore formation, which allowed for the efflux of intracellular materials, ultimately leading to cell death.

According to Akkam (2016), antifungal peptides are cationic and possess positive net charges of +1 – +5. For example, the antifungal peptide Heliomicin, isolated from the worm *Heliiothis virescens* has a net charge of +1. Heliomicin exhibits an inhibitory effect on two pathogenic fungi, *C. albicans* and *Candida neoformans* (Lamberty et al. 2001). Maximin 1 has a net charge of +3 and inhibits *C. albicans* growth (Lai et al. 2002). Apart from peptide net charge, peptide hydrophobicity is also required for peptide membrane interaction and membrane permeabilization. peptide hydrophobicity is also control the peptide portioning into the phospholipid layer. Previous study proposed that the hydrophobic value of vast majority of antifungal proteins are in the range of 30% and 60% (Akkam, 2016). The calculated hydrophobicity of this bacteriocin was 42%. Overall, it is assumed that these two properties of bacteriocin produced by *Bacillus* sp. Sh10 had a great impact on its function which is pore formation.

CONCLUSION

In conclusion, the present antifungal bacteriocin is a novel bacteriocin. Furthermore, elucidation of the structure and the genetic determination of this

bacteriocin can provide insights into its further classification and functional relationship to its mode of action.

Acknowledgements: This work was supported by University Kebangsaan Malaysia (Faculty of Science and Technology).

REFERENCES

- Adebayo, C.O., & Aderiyi, B.I. (2011). Suspected mode of antimycotic action of brevicin SG1 against *Candida albicans* and *Penicillium citrinum*. *Food Control*, 22 (12), 1814-1820. <http://doi:10.1016/j.foodcont.2011.04.013>
- Akkam, Y. (2016). A Review of Antifungal Peptides: Basis to New Era of Antifungal Drugs. *Jordan Journal of Pharmaceutical Sciences*, 9 (1), 51-75.
- Amel, M.S., Sohair, R.F., & Wessam, A.M. (2015). Therapeutic efficacy of chitosan against invasive candidiasis in mice. *The Journal of Basic & Applied Zoology*, 72, 163-172. <http://doi:10.1016/j.jbaz.2014.12.001>
- Barboza-Corona, J. E., Vazquez-Acosta, H., Bideshi, D. K., & Salcedo-Hernandez, R. (2007). Bacteriocin-like inhibitor substances produced by Mexican strains of *Bacillus thuringiensis*. *Archives Microbiology*, 187 (2), 117-126. <http://doi:10.1007/s00203-006-0178-5>
- Benitez, L.B., Caumo, K., Brandelli, A., & Rott, M.B. (2011). Bacteriocin-like substance from *Bacillus amyloliquefaciens* shows remarkable inhibition of *Acanthamoeba polyphaga*. *Parasitology Research*, 108 (3), 687-691. <http://doi:10.1007/s00436-010-2114-5>
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72 (1-2), 248-54. <http://doi:10.1006/abio.1976.9999>
- Cherif, A., Chehimi, S., Limem, F., Hansen, B. M., Hendriksen, N. B., Daffonchio, D., & Boudabous, A. (2003). Detection and characterization of the novel bacteriocin entomocin 9, and safety evaluation of its producer, *Bacillus thuringiensis* subsp. *entomocidus* HD9. *Journal of Applied Microbiology*, 95 (5), 990-1000. <http://doi:10.1046/j.1365-2672.2003.02089.x>
- Cherif, A., Ouzari, H., Daffonchio, D., Cherif, H., Ben Slama, K., Hassen, A., ... & Boudabous, A. (2001). Thuricin 7: a novel bacteriocin produced by *Bacillus thuringiensis* BMG1.7, a new strain isolated from soil. *Journal of Applied Microbiology*, 32 (4), 243-247. <http://doi:10.1046/j.1472-765x.2001.00898.x>
- Cladera-Olivera, F., Caron, G. R., & Brandelli, A. (2004). Bacteriocin-like substance production by *Bacillus licheniformis* strain P40. *Letters in Applied Microbiology*, 38 (4), 251-256. <http://doi:10.1111/j.1472-765x.2004.01478.x>
- Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, 3 (10), 777-788. <http://doi:10.1038/nrmicro1273>
- Crupper, S.S., & Iandolo, J.J. (1996). Purification and partial characterization of a novel antibacterial agent (Bac1829) produced by *Staphylococcus aureus* KSI1829. *Applied Environmental Microbiology*, 62 (9), 3171-3175.
- De la Fuente-Salcido, N., Alanís-Guzmán, M.G., Bideshi, D.K., Salcedo-Hernández, R., Bautista-Justo, M., & Barboza-Corona, J.E. (2008). Enhanced synthesis and antimicrobial activities of bacteriocins produced by Mexican strains of *Bacillus thuringiensis*. *Archives Microbiology*, 190 (6), 633-640. <http://doi:10.1007/s00203-008-0414-2>
- Gavanji, S., Zaker, S.R., Nejad, Z.G., Bakhtari, A., Bidabadi, E.S., & Larki, B. (2015). Comparative efficacy of herbal essences with amphotericin B and ketoconazole on *Candida albicans* in the in vitro condition. *Integrative Medicine Research*, 4 (2), 112-118. <http://doi.org/10.1016/j.imr.2015.01.003>
- Gudlaugsson, O., Gillespie, S., LEE, K., Vande Berg, J., HU, J., MESSER, S., ... & Diekema, D. (2003). Attributable mortality of nosocomial candidemia, revisited. *Clinical Infectious Diseases*, 37 (9), 1172-1177. <http://doi:10.1086/378745>
- Harris, E.L.V., & Angal, S. (1989). Protein purification Methods: A practical Approach. IRL Press, Oxford, pp. 210-290.
- Hoover, D.G., & Harlander, S.K. (1993). Screening Methods for Detecting Bacteriocin Activity. In: Hoover, D.G., & Steenscr. L.R., (Eds.), Bacteriocins of Lactic Acid Bacteria. Academic Press, San Diego, pp. 23-39.
- Hyronimus, B., Le Merrec, C., & Urdaci, M.C. (1998). Coagulin: A bacteriocin-like inhibitory substance produced by *Bacillus coagulans* 14. *Journal of Applied Microbiology*, 85 (1), 42-50. <http://doi:10.1046/j.1365-2672.1998.00466.x>
- Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *Federation of European Microbiological Societies Microbiol Review*, 12 (1-3), 39-85. <http://doi:10.1111/j.1574-6976.1993.tb00012.x>
- Laemmlli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227 (5259): 680-685. <http://doi:10.1038/227680a0>
- Lai, R., Zheng, Y.T., Shen, J.H., Liu, G.J., Liu, H., Lee, W.H., ... & Zhang, Y. (2002). Antimicrobial peptides from skin secretions of Chinese red belly toad *Bombina maxima*. *Peptides*, 23 (3), 427-435. [http://doi:10.1016/s0196-9781\(01\)00641-6](http://doi:10.1016/s0196-9781(01)00641-6)
- Lamberty, M., Caille, A., Landon, C., TassinMoindrot, S., Hetru, C., Bulet, P., & Vovelle, F. (2001). Solution structures of the antifungal heliomicin and a selected variant with both antibacterial and antifungal activities. *Biochemistry*, 40 (40), 11995-12003. <http://doi:10.1021/bi0103563>

- Leroy, F., De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*, 15 (4), 67-78. <http://doi:10.1016/j.tifs.2003.09.004>
- Naclerio, G., Ricca, E., Sacco, M., & de Felice, M. (1993). Antimicrobial activity of a newly identified bacteriocin of *Bacillus cereus*. *Applied and Environmental Microbiology*, 59 (12), 4313-4316.
- Ostrosky-Zeichner, L.L., Casadevall, A., Galgiani, J.N., Odds, F.C., & Rex, J.H. (2010). An insight into the antifungal pipeline: selected new molecules and beyond. *Nature Reviews Drug Discovery*, 9 (9), 719-727. <http://doi:10.1038/nrd3074>
- Paik, H. D., Bae, S. S., & Pan, J. G. (1997). Identification and partial characterization of tochicin, a bacteriocin produced by *Bacillus thuringiensis* subsp. *tochigiensis*. *Journal of Industrial Microbiology & Biotechnology*, 19 (4), 294-298. <http://doi:10.1038/sj.jim.2900462>
- Pridham, T.G., & Gottlieb, D. (1948). The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *Journal of Bacteriology*, 56 (1), 107-114.
- Roongsawang, N., Washion, K., & Morikawa, M. (2011). Diversity of nonribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. *International Journal of Molecular Sciences*, 12 (1), 141-172. <http://doi:10.3390/ijms12010141>
- Seuk-Hyun, K., & Cheol, A. (2000). Bacteriocin production by *Lactococcus lactis* KCA 2386 isolated from white kimchi. *Food Science and Biotechnology*, 9, 263-269.
- Shayesteh, F., Ahmad, A., & Usup, G. (2014). Bacteriocin production by a marine strain of *Bacillus* sp. Sh10: Isolation, screening and optimization of culture condition. *Biotechnology*, 13 (6), 273-281. <http://doi:10.3923/biotech.2014.273.281>
- Shayesteh, F., Ahmad, A., & Usup, G. (2015). Partial Characterization of an Anti-*Candida albicans* Bacteriocin Produced by a Marine Strain of *Bacillus* sp., Sh10. *Advance Journal of Food Science and Technology*, 9 (9), 664-671. <http://doi:10.19026/ajfst.9.1757>
- Shelburne, C.E., An, F.Y., Dholpe, V., Ramamoorthy, A., Lopatin, D.E., & Lantz, M.S. (2007). The spectrum of antimicrobial activity of the bacteriocin subtilisin A. *Journal of Antimicrobial Chemotherapy*, 59 (2), 297-300. <http://doi:10.1093/jac/dkl495>
- Whitford, W.F., McPherson, M.A., Foster, R.J., & Teather, R.M. (2001). Identification of bacteriocin-like inhibitors from rumen *Streptococcus* spp. and isolation and characterization of Bovicin 255. *Applied Environmental Microbiology*, 67(2), 569-574. <http://doi:10.1128/AEM.67.2.569-574.2001>