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Original Article

بررسی مکانیسم اثر خاصیت ضد قارچی باکتریوسین تولید شده توسط *Bacillus* sp. Sh54

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چکیده

سابقه و هدف: باکتریوسین‌ها پپتیدهای ضد میکروبی هستند که توسط باکتری‌های مختلف تولید می‌شوند و به عنوان عامل درمانی مورد استفاده قرار می‌گیرند. هدف از این مطالعه بررسی مکانیسم اثر ضد میکروبی باکتریوسین تولید شده توسط باکتری دریایی Bacillus sp. Sh10، بر علیه پاتوژن Candida albicans ATCC 10231 می‌باشد.

مواد و روش‌ها: مکانیسم ضد میکروبی باکتریوسین با استفاده از روش‌های زنده مانی سلول، میزان خروج مواد جاذب UV، نمک‌های معدنی، K+ ATP از سلول و همچنین میکروسکوپ الکترونی روبشی و عبوری مورد بررسی قرار گرفت.

یافته‌ها: افزودن میزان ۱ × MIC باکتریوسین به سوسپانسیون سلولی C. albicans تعداد سلول‌های زنده را حدود ٤ واحد لگاریتمی طی ۱۰ ساعت کاهش داد. همچنین، با توجه به خروج مواد جاذب UV، نمک‌های معدنی، K+ ATP از سلول‌های C. albicans مشخص گردید که باکتریوسین مورد نظرباعث کشتن سلول‌های قارچی گردیده است. علاوه بر این سلول‌های تیمار شده با باکتریوسین نسبت به پروپیدیوم یدید نفوذپذیر گشتند. مشاهدات میکروسکوپ الکترونی روبشی و عبوری تغییرات زیادی را در مرفولوژی سلول از جمله سطح چروکیده، دیواره سلولی ناپیوسته و پاره شده به همراه لایز شدن سلول‌ها نشان داد.

نتیجه‌گیری: نتایج به‌دست آمده در مطالعه حاضر نشان داد که باکتریوسین با غشای سیتوپلاسمی سلول‌های C. albicans برهم کنش داشته و منجر به تشکیل منافذ می‌شود، که این تغییرات منجر به خروج مواد داخل سلولی و در نهایت باعث مرگ سلول می‌شود.

واژگان کلیدی: باکتری‌های دریایی، باکتریوسین، ضد قارچ، کاندیدا آلبیکنس، باسیلوس.

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Original Article

Mechanism of action of an antifungal batercin produced by a marine *Bacillus* sp. Sh10

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Abstract

Background and objective: bacteriocins are antimicrobial peptides produced by different bacteria and can be applied as a therapeutic agent. The aim of this study was to investigate the mode of action of broad-spectrum bacteriocin produced by a marine Bacillus, strain Sh10, on *Candida albicans* ATCC 10231.

Materials and Methods: Cell viability assay, determination of UV-absorbing materials, K+, inorganic phosphate, ATP, and LIVE/DEAD cell viability assay as well as scanned and transmission electron microscopy were used to investigate the mode of action of bacteriocin.

Results: The addition of 1 × MIC of bacteriocin to a cell suspension of *C. albicans* decreased the number of viable cells by about 4 log units over a period of 10 hours. It displayed a fungicidal mode of action with a massive leakage of K+ ions, inorganic phosphates, ATP, and UV-absorbance materials, leading to cell lysis. In addition, the permeability of *C. albicans* treated cells to propidium iodide was observed. The electron microscopic observations of treated cells indicated several modifications in cell morphology such as wrinkled surface, discontinuous and ruptured cell wall with concomitant lysis.

Conclusion: The data obtained in the current study demonstrated that the present bacteriocin interacted with the cytoplasmic membrane of *C. albicans* cells, resulting in pore formation, resulting in the efflux of interacellular materials that exhibit a fungicidal effect.

Key words: Marine bacteria, Bacteriocin, Antifungal, *Candida albicans*, *Bacillus* sp.

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Introduction

Antimicrobial agents are among the greatest discoveries in medicine in the early 20th century. Before their accidental discovery, millions of people died from simple bacterial infections. However, these agents have been overused and misused; as a consequence, resistant microorganisms have inadvertently evolved. Drug resistance has been observed in various microorganisms, such as *Candida albicans* (1). *C. albicans* is an opportunistic fungal pathogen that resides as a commensal in normal individuals but causes infections under altered physiological and pathological conditions, such as infancy, pregnancy,

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diabetes, prolonged broad-spectrum antibiotic administration, steroidal chemotherapy, and acquired immune deficiency syndrome (AIDS) (2,3). *C. albicans* accounts for more than 90% of a series of fungal infections (4,5). Patients infected with Candida display higher morbidity and mortality, stay longer in hospitals, and spend higher costs of medical care than uninfected patients do (6-8). Although antifungal agents have been developed for over 50 years, the management of Candida infections faces serious problems, including limited number of effective antifungal drugs, resistance of Candida to commonly used antifungal drugs, relapse of Candida infections, and high cost of antifungal drugs (1,9). As a result, novel therapeutic solutions other than the current antifungal therapies are an urgent need. Bacteriocins have attracted attention because of their high diversity, broad spectrum activity, non-toxicity, heat and pH stability, and relatively cost-effective production (10-12).

Bacteriocins, the most potent of the antimicrobial substances produced by a group of bacteria are ribosomally-synthesized proteins with antimicrobial activity (13). Bacteriocins generally exert their antimicrobial action by interfering with the cell wall or the membrane of target organisms, either by inhibiting cell wall biosynthesis or causing pore formation, subsequently resulting in cell death and cell lysis (14).

According to our previous study, the bacteriocin produced by *bacillus* sp. Sh10 inhibited the growth of many food spoilage and human pathogens with a maximum inhibitory activity against *C. albicans* ATCC 10231 and due to its strong physico-chemical stability, this peptide has a good potential to be developed as antimicrobial agent for food and clinical application (15,16). Therefore, for any practical application of antimicrobial compounds, there is a need to study their mode of action. The suposed mechanism of antagonism of bacteriocin produce from a marine *Bacillus* Sh10 on *C. albicans* cells is reported in this study.

Materials and methods

1. Preparing of bacteriocin fraction, detections and assay: The bacteriocin-producing isolate *Bacillus* sp. Sh10 was isolated from the carpet clam (Paphia textile) using marine agar medium and was cultured in optimized medium containing inorganic salts (17), 1% glucose, 2% tryptone, and 2% sodium chloride (NaCl). The culture medium was adjusted to pH 8.0 and bacteria were incubated at 30 °C under aerobic condition (15). After 30 h, the cells were removed by centrifugation at 3000 × g for 30 min at 4 °C. The obtained supernatant was then passed through a membrane filter (0.22 µm) and was precipitated with ammonium sulfate at 80% of saturation for 24 h at 4 °C with gentle stirring. The precipitated protein was extracted by centrifugation at 3000 × g for 30 min. The obtained protein was dissolved in phosphate buffer (0.1 M, pH 7.0), dialyzed through a 2 kDa cut-off dialysis membrane (Sigma) against the same buffer at 4 °C for 24 h (18), and designed as ammonium sulfate fraction of bacteriocin.

The antimicrobial activity of bacteriocin against *C. albicans* ATCC 10231 was determined using the spot-on-lawn method (19). In brief, 10 µL of ammonium sulfate fraction of bacteriocin was spotted onto the surface of trypton soy agar overlaid with 1.5 × 108 CFU/mL cells of the *C. albicans* and then incubated at 30 °C. After 48 h of incubation, the zone of growth inhibition was observed.

Preparing of *C. albicans* cell suspension

C. albicans ATCC 10231 were obtained from the Marine Microbial Culture Collection in Marine Microbiology & Biotechnolgy lab 3162/Universiti Kebangsaan Malaysia (UKM). For all experiments, *C. albicans* cells were grown to the mid-exponential phase at 30 °C in trypton soy broth and centrifuged at 3000 × g for 10 min. The cells were washed twice with 5 mmol L−1 sodium phosphate buffer (pH 7.0) and then resuspended in the same medium. Turbidity was adjusted to 0.5 McFarland (1.5 × 108 CFU/mL).

2. Determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) against C. albicans cells: The MIC and MFC of gel filtrate active fraction of bacteriocin against planktonic *C. albicans* cells were determined using the microdilution method (20). In brief, 50 µL of *C. albicans* cell suspension was added to an equal volume of ammonium sulfate fraction of bacteriocin with different concentrations (0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µg). The diluted suspensions were then incubated for 48 h at 30 °C. The MIC was the lowest concentration of bacteriocin that prevented visible turbidity after 24 h of incubation in trypton soy broth. After MICs were determined, 10 µL of the solutions from the clear wells was plated onto trypton soy agar and incubated at 30 °C overnight. The MFC were defined as the lowest concentration of bacteriocin required to eradicate *C. albicans* cells on the media.

3. Effects of bacteriocin on the viability of C. albicans: To determine *C. albicans* cell viability, the ammonium sulfate fraction of bacteriocin was added to the *C. albicans* cell suspension to obtain a final concentration of 1 × MIC. The samples were incubated at 30 °C for 10 h. The inhibitory effect of bacteriocin on growth of *C. albicans* was measured in terms of the change in OD600 and cell viability at 1-h intervals. The number of viable cells in tryptone soy agar plates was determined as colony forming unit (CFU) after 72 h of incubation at 30 °C. the culture of *C. albicans* without bacteriocin was used as a control sample.

4. Effects of bacteriocin on leakage of UV-absorbing materials: The release of UV-absorbing materials (protein and nucleic acid) was used as a lysis index of the indicator organism exposed to bacteriocin. Briefly, bacteriocin (1 × MIC) was added to *C. albicans* cell suspension at 30 °C for 4 h. The treated cells were harvested at 6000 × g for 20 min at 4 °C every 30 min, and the supernatant was filtered through a sterile nitrate cellulose membrane (0.22 µm). The absorbance of the cell-free supernatant was determined at 260 and 280 nm to assess the concentration of nucleic acid and protein, respectively (21,22). The cell suspension treated with phosphate buffer was used as a control sample.

5. Measurement of K+ and inorganic phosphate contents of cells: Intracellular and extracellular K+ content was conducted by addition of bacteriocin (1× MIC) to *C. albicans* cell suspension. Gel filtration active fraction of bacteriocin was added to a final concentration of 1× MIC. Samples (1 mL) were taken at various time intervals and then immediately chilled on ice. The cells were subsequently obtained through centrifugation at 10,000 × g for 7 min at 0 °C. The supernatant was removed and stored for the determination of extracellular K+. Moreover, the cell pellet was resuspended in 1 mL of 5% (w/v) trichloroacetic acid and then frozen overnight at 20 °C. The samples were then thawed and incubated at 95 °C for 10 min. Demineralized

water (4 ml) was added into each sample and subsequently centrifuged at 10,000 × g for 15 min.

The supernatant was retained for intracellular K+ determination. K+ concentration in the samples was determined by flame photometry (M7D). Inorganic phosphate was determined in the supernatants by the reduction of the formed phosphomolybdate complex with ascorbic acid (23). The *C. albicans* culture without bacteriocin was used as control.

6. Measurement of intracellular and extracellular ATP: To determine whether bacteriocin affects the internal adenosine 5′-triphosphate (ATP) level in *C. albicans* cells, the intracellular and extracellular concentration of ATP were measured by fluorescent spectrophotometer (Whaltman, MA, USA) according to the method described by Chen and Montville (24) with some modifications. An ATP bioluminescence assay kit (Promega) was used. *C. albicans* cells were prepared and kept on ice until use. In order to energise the cells prior to ATP measurements, they were resuspended to half of the original volume in 50 mM 2-(N-morpholino) ethane-sulfonate (MES) buffer and incubated for 20 min. After treating the cells with bacteriocin (1× MIC), the total and extracellular ATP levels were measured using the bioluminescence method. Intracellular ATP was calculated by subtracting external ATP from the total ATP. The assays were calibrated using a standard curve obtained by measuring the bioluminescence of ATP solutions of known concentrations, and the ATP levels were expressed as ng/ml of cells (dry weight). The *C. albicans* culture without bacteriocin was used as control.

7. LIVE/DEAD cell viability assay: To confirm the fungicidal activity of bacteriocin, *C. albicans* cells were stained by the LIVE/DEAD Abnova cell viability staining kit KA0901 in accordance with the manufacturer's instructions. In brief, *C. albicans* cells suspension were treated with 1 × MIC of bacteriocin. After 2 h, the final culture was stained using the cell viability kit and viewed under a fluorescent microscope (Olympus IX81). *C. albicans* culture without bacteriocin was used as a control sample.

8. Electron microscopy observation: Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) were used to observe the effect of bacteriocin on *C. albicans* cell morphology.

For this purpose, ammonium sulfate fraction of bacteriocin was added to *C. albicans* cell suspension to obtain a final concentration of 1 × MIC, and the samples were incubated at 30C. After 2 h of incubation, the control and treated cells were harvested by centrifugation, washed with phosphate buffer, and immediately fixed with 2% (v/v) glutaraldehyde in phosphate buffer. Serial dehydration using 30% to 100% of ethyl alcohol was performed followed by critical point drying (CPD).

The samples were sputtered with gold by using a polaron coater and viewed under a Philips XL 300 SEM. For TEM observation the harvested cells were fixed by 2% (v/v) glutaraldehyde followed by post-fixing with 1% (v/v) osmium tetroxide for 1 h. The post-fixed samples were serially dehydrated using 30% to 100% ethyl alcohol.

The samples were then embedded in epoxy resin; thin sections (70–80 nm) were obtained using a microtome and were mounted onto 300-mesh copper grides.The sections were stained with 2% (v/v) alcoholic uranyl acetate and 3% (v/v) alkaline lead citrate, washed gently with distilled water, and observed using a Philips CM12 TEM.

9. Statistical analysis: All the assays in this study were carried out in triplicate. Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements using Microsoft Excel software.

Result

1. Determination of MIC and MFC of bacteriocin against planktonic cell of C. albicans: The MIC and MFC of gel filtrate ammonium sulfate fraction of bacteriocin produced by isolate Sh10 were 0.5 and 1 µg/mL, respectively.

2. Effect of bacteriocin on the viability of C. albicans cells: The addition of bacteriocin to a cell suspension of *C. albicans* decreased the number of viable cells by about 4 log units compared with the control over a period of 10 h. Thus, bacteriocin has fungicidal effect on C. albicans. However, the OD600 of bacteriocin-treated cells remained constant during 3 h of incubation and start to decrease after 3 h. This finding demonstrated that bacteriocin induced cell lysis after cell death. By contrast, the OD600 of the untreated sample increased from 0.125 to 2.35 nm during 10 h of incubation (Figure 1).

3. Effect of bacteriocin on leakage of UV-absorbing materials: C. albicans treatment with purified bacteriocin resulted in an increase of extracellular UV-absorbing materials at OD260, whereas the OD260 of untreated sample increased after 80 min incubation (Figure 2A). OD280 also increased after treatment of *C. albicans* cells with bacteriocin for 4 h (Figure 2B). By contrast, the OD280 of untreated sample increased over the same time of incubation. These findings indicated that the cells treated with bacteriocin become permeable to UV-absorbing materials at 260 and 280 nm.

4. Measurement of K+ and inorganic phosphate contents of cells: In the absence of bacteriocin, *C.albicans* cells had an intracellular concentration of K+, approximately 34 µmol L−1. However, cells treated with bacteriocin showed a detectable loss of intracellular K+.

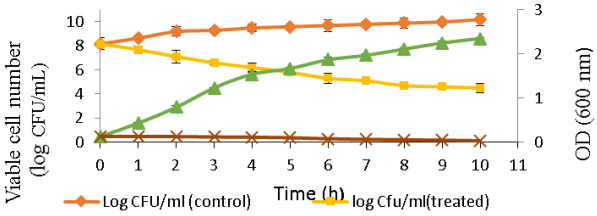

Figure 1. Effect of gel filtrate fraction of bacteriocin on mid-exponential phase, *C. albicans* ATCC 10231.

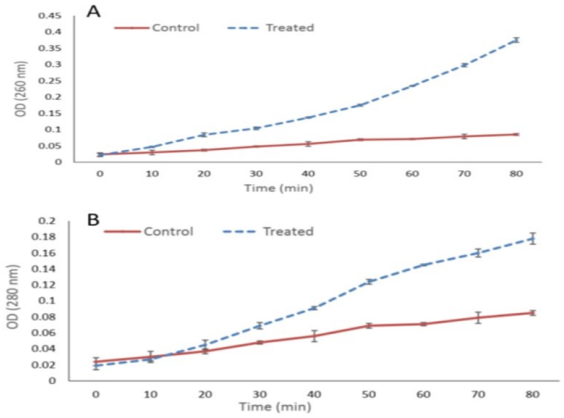

Figure 2. Effect of ammonium sulfate fraction of bacteriocin on the leakage of UV-absorbing materials.

concentration of extracellular K+ increased in the treated sample, indicating that bacteriocin had induced massive leakage of K+ from *C. albicans* cells. The release of K+ was immediate and after 10 min of treatment with bacteriocin (Figure 3). Moreover, after addition of bacteriocin to *C. albicans* cells, intracellular phosphate decreased from 70 µmol L−1 to 29 µmol L−1, while extracellular phosphate increased from 0 to 50 µmol L−1. The concentrations of extracellular and intracellular phosphate from untreated cells remained constant during the assay at 0 and 70 µmol L−1, respectively (Figure 3).

5. Measurement of intracellular and extracellular ATP: Untreated *C. albicans* cells maintained their intracellular and extracellular ATP levels of approximately 23 and 11 ng/mL, respectively. Treatment of *C. albicans* cells with 1×MIC of bacteriocin increased the extracellular ATP.

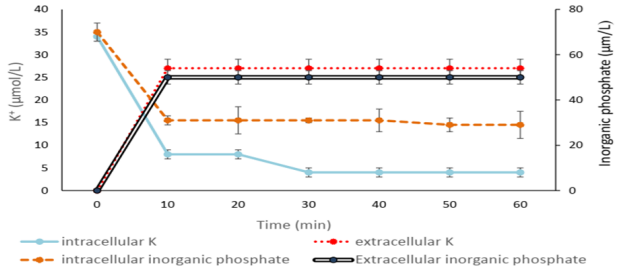

Figure 3. Intracellular and extracellular K^+ ions and inorganic phosphate level after treatment with ammonium sulfate fraction of bacteriocin.

level of cells from 11 ng/mL to 26 ng/mL within 50 min. In addition, bacteriocin caused a gradual reduction in intracellular ATP level from 23 ng/mL to 10 ng/ml over the period of incubation. Measurement of extracellular and intracellular ATP content of treated cells indicated that the bacteriocin resulted in an efflux of ATP from inside of the cells (Figure 4).

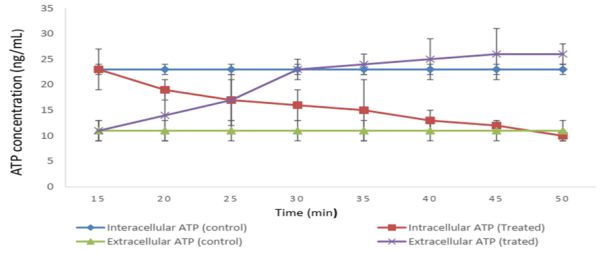

Figure 4. Intracellular and extracellular ATP levels after treatment with ammonium sulfate fraction of bacteriocin.

6. LIVE/DEAD cell viability assay: Upon staining of C.albicans cells using a commercial viability kit, most bacteriocin-treated cells demonstrated an orange-red color, thereby indicating altered cell membrane integrity, whereas most cells in the control exhibited green fluorescence (Figure5).

Thus, bacteriocin is capable of changing the integrity of the cell membrane, possibly by promoting the development of membrane lesions.

7. Electron microscope observation: SEM observation indicated that *C. albicans* cells treated with bacteriocin showed damaged cell surface compared with untreated cells. Several damages in the surface of bacteriocin-treated cells were observed, including roughness, indentation, and wrinkling of the cells, as well as oozing out of intracellular material. In addition, few completely lysed cells were visible (Figure 6). These observations clearly suggested the damaging effects of bacteriocin on the surface layers of cells. TEM analysis showed discontinuity, rupture, and collapse of cell wall in bacteriocin-treated *C. albicans* cells, as shown in Figures 7. Completely lysed cells were also observed. These observations confirmed the fungicidal effect of bacteriocin on *C. albicans* cells.

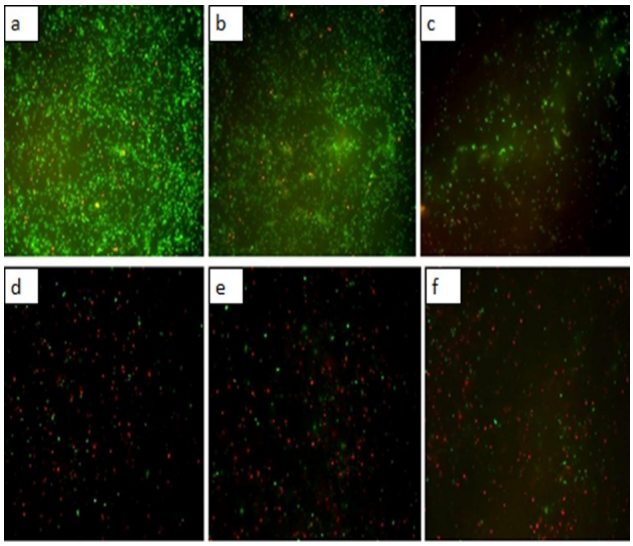

Figure 5. Fluorescent micrograph of *C. albicans* cells; a, b, and c, control cells showing green colour (live cells); d, e, and f, bacteriocin treated cells showing red colour (dead cells) (× 60).

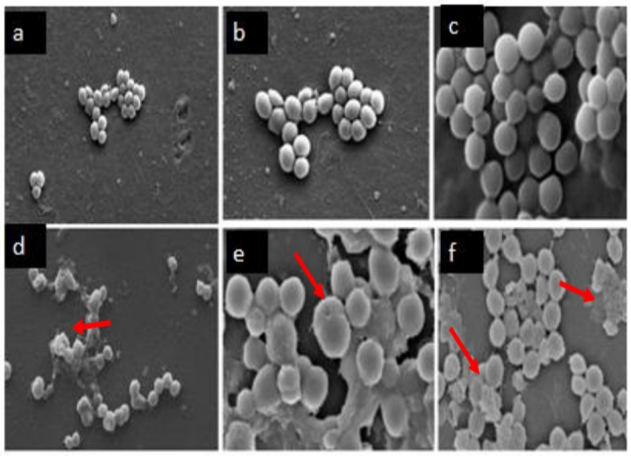

Figure 6. SEM micrograph of planktonic cells of C. albicans; a, b, and c, control cells; d, e, and f, bacteriocin treated cells. Magnifications 3000 X (a, d) and 7000 X (b, c, e, f). Red arrow showing the damaged cells.

Discussion

Increases in the leakage of potassium ions and K^+ ions indicate a ruptured cell membrane. Membrane permeability and surface characteristic parameters were used to determine the mode of action of bacteriocin produced by marine bacteria, *Bacillus* sp. Sh10, against the indicator pathogen *C.albicans*. The SEM study confirmed the fungicidal effect of bacteriocin on *C. albicans* cells which revealed several extracellular changes in the cell wall (roughness, indentation and wrinkling), as is reported for synthetic peptides VS2 and VS3 (25) and plantaricins E/F and J/K (2).

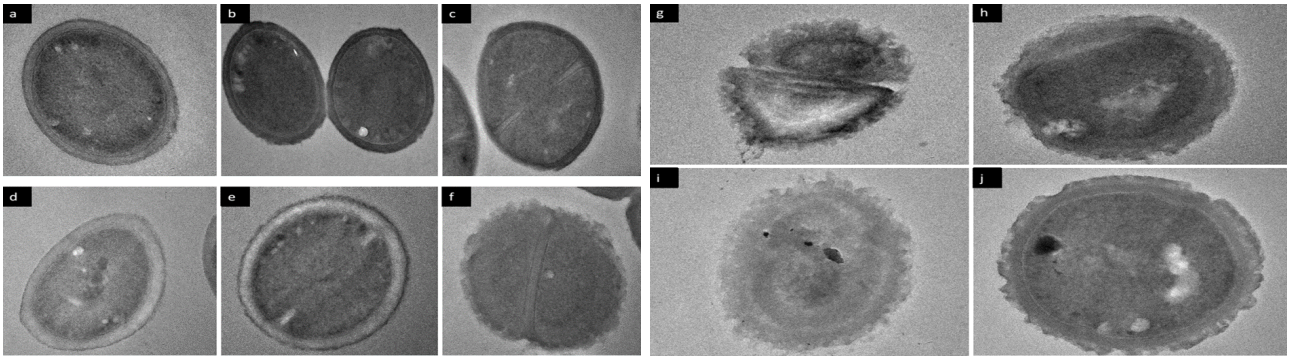

Figure 7. TEM observation of *C. albicans* cell. (a-e) untreated control cells; (f-j) bacteriocin treated cells. Magnification 17000X (a, b, g); 22000 X (c, d, e, f, h, i, j).

In TEM analysis, discontinuous and ruptured cell wall observed could be due to altered permeability of the cell membrane which could provoke the osmotic imbalance leading to indentations of the cell wall in treated cells, as also reported by Sharma and Srivastava (26). Similarly broken and damaged cell wall and membrane was also observed for de novo designed cationic peptides, VS2 and VS3 against Candida (25).

The results of cell viability assay suggest that this antimicrobial peptide has a fungicidal effect against C. albicans, based on the observed decline in the number of living cells of *C. albicans* after the addition of bacteriocin. A fungicidal effect for this compound is also supported by the decrease in OD600, which indicated that the cells of indicator strains had lysed . The loss in viability of the indicator strain is linked to bacteriocin induced plasma membrane pores, which allowed extrusion of the cell content and consequently cell lysis.

ATP is used for many cell functions including transport work moving substances across cell membranes which might be a potential target parameter to understand the mode of action of antimicrobial agents. The results of our study on the extracellular and intracellular ATP concentration showed an increasing rate of extracellular ATP and decreasing rate of

intracellular ATP concentrations after *C. albicans* cells exposed to bacteriocin. This might occur due to significant impairment in membrane permeability of the target cells by bacteriocin, which caused the ATP leakage through defective cell membrane. Similar findings on this phenomenon have also been reported for various bacteriocins (27,28). In addition, Zhou et al. (22) reported the exposure of *Listeria monocytogenes* cells to pentocin 31-1 resulted in decreased level of intracellular ATP while increased the level of intracellular ATP. Moreover, the significant reduction in intracellular ATP can be resulted due to the loss of inorganic phosphate across the compromised high permeable cell membrane or in virtue of the efforts made by the cell to recover the electrochemical gradient by proton motive f orce (PMF) driven by the ATPase, an increased ATP hydrolysis is established (24). To allow permeation of large molecules, such as ATP, it is likely that several bacteriocin molecules act to form multipeptide pore complexes (29).

A fluorescent technique was used to confirm the damage of bacteriocin-treated C. albicans cell membrane. A fluorescent technique involving two fluorescent dyes, SYTO®9 (a green fluorescent nucleic acid stain that labels all cells in a population) and propidium iodide (PI, a red fluorescent nucleic acid stain that only penetrates cells with damaged membranes).

When SYTO®9 and PI are used in combination, microorganisms with intact cell membranes stained fluorescent green, whereas those having damaged membranes stain fluorescent red. As shown in figure 5, most bacteriocin-treated cells demonstrated a red color which indicating bacteriocin is able to damage cytoplasmic membrane of sensitive cells.

Exposure of *C. albicans* cells to bacteriocin caused large leakage of UV absorbing materials (nucleic acid and protein) from the treated cells. The release of UV-absorbing materials was generally used as an index of non -selective pore formation (22). Larger leakage of intracellular UV-absorbing materials at 260 and 280 nm was also observed in E. coli ATCC 25922 after treated with sakacin C2 which was due to formation of pores in the cytoplasmic membrane (30).

The microorganism plasma membrane provides a permeability barrier to the passage of small ions such as potassium ions which are necessary electrolytes, facilitating cell membrane functions and maintaining proper enzyme activity. This impermeability to small ions is maintained and even regulated by the structural and chemical composition of the membrane itself. Increases in the leakage of potassium ions will indicate a disruption of this permeability barrier. Treatment of C. albicans cells with bacteriocin resulted in the release of almost all detectable internal inorganic phosphate as well as K+, this release occurred almost immediately through the pores formation. The loss of these essential ions may be a consequence of the ATP hydrolysis that occurs as a result of bacteriocin action, presumably due to consumption of the available ATP in a futile effort to re-accumulate inorganic phosphate and K+ by ATP-dependent uptake systems.

In conclusion, these data suggest that the present bacteriocin interacted with the cytoplasmic membrane of *C. albicans* cells, resulting in pore formation. The pores lead to leakage of cyptoplasmic contents and death of fungi cells. Further evaluation in in-vivo cultures is required to determine whether these

findings can be exploited in treating *C. albicans* infections.

Acknowledgement

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Klepser ME. Antifungal resistance among Candida species. Pharmacotherapy. 2001; 21 (8 Pt 2): 124-132.
2. Mackenzie DWR, Cauwenberg G, Van Cutsem J, Drouhet E, Dupont B. Mycoses in AIDS patients: An overview.1990; New York, Plenum Press.
3. Friedman S, Richardson SE, Jacobs SE, O'Brien K. Systemic Candida infection in extremely low birth weight infants: Short term morbidity and neuro developmental outcome. The Pediatr Infect Dis J. 2000; 19 (6): 499-504.
4. Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on Candida species. Clin Infect Dis. 1995; 20 (6): 1526-1530.
5. Douglas LJ. Candida biofilms and their role in infection. Trends Microbiol. 2003; 11 (1): 30-36.
6. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20 (1): 133-63.
7. Arendrup MC, Sulim S, Holm A, Nielsen L, Nielsen SD, Knudsen JD, Drenck NE, Christensen JJ, Johansen HK. Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. J Clin Microbiol. 2011; 49 (9): 3300-3308.
8. Lai CC, Wang CY, Liu WL, Huang YT, Hsueh PR. Time to positivity of blood cultures of different Candida species causing fungaemia. J Med Microbiol. 2012; 61 (Pt 5):701-704.
9. Khan ZU, Chandy R, Metwali KE. Candida albicans strain carriage in patients and nursing staff of an intensive care unit: a study of morphotypes and resistotypes. Mycoses. 2003; 46 (11 -12): 476-486.
10. Riley MA, Wertz JE. Bacteriocin diversity: ecological and evolutionary perspectives. Biochimie. 2002; 84 (5-6): 357-364.

11. Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: advantages and limits. Curr Med Chem. 2011; 18 (2): 256-279.
12. Okuda K, Zendo T, Sugimoto S, Iwase T, Tajima A, Yamada S, Sonomoto K, Mizunoe Y. Effects of bacteriocins on methicillin-resistant Staphylococcus aureus biofilm. Antimicrob Agents Chemother. 2013; 57 (11): 5572-5579.
13. Savadogo A, Ouattara CAT, Basole IHN, Traore SA. Bacteriocins and lactic acid bacteria: a mini-review. Afr J Biotechnol. 2006; 5 (9): 678-683.
14. O' Sullivan LO, Ross PP, Hill C. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie. 2002; 84 (5-6): 593-604.
15. Shayesteh F, Ahmad A, Usup G. Bacteriocin production by a marine strain of *Bacillus* sp. Sh10: Isolation, screening and optimization of culture condition. Biotechnol. 2014; 13 (6): 273 -281.
16. Shayesteh F, Ahmad A, Usup G. Partial Characterization of an Anti-Candida albicans Bacteriocin Produced by a Marine Strain of *Bacillus* sp., Sh10. Adv J Food Sci Technol. 2015; 9 (9): 664-671.
17. Pridham TG, Gottlieb D. The utilization of carbon compounds by some actinomycetales as an aid for species determination. J Bacteriol. 1948; 56 (1): 107-114.
18. Rajaram, G, Manivasagan P, Thilagavathi B, Saravanakumar A. Purification and characterization of a bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. Adv J Food Sci Technol. 2010; 2 (2): 138-144.
19. Seuk-Hyun K, Cheol A. Bacteriocin production by Lactococcus lactis KCA 2386 isolated from white kimchi. Food Sci Biotechnol. 2000; 9: 263-269.
20. Clinical and Laboratory Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th edn. 2003; CLSI Document M7-A6: Wayne.
21. Motta AS, Flores FS, Souto AA, Brandelli A. Antibacterial activity of a bacteriocin-like substance produced by *Bacillus* sp. P34 that targets the bacterial cell envelope. Antonie Van Leeuwenhoek. 2008; 93 (3): 275-284.
22. Zhou K, Zhou W, Li P, Liu G, Zhang, J. Mode of action of pentocin 31-1: An antilisteria bacteriocin produced by *Lactobacillus pentosus* from Chinese traditional ham. Food Control. 2008; 19 (8): 817-22.
23. Ames BN. Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol. 1966; 8: 115-118.
24. Chen Y, Montville TJ. Efflux of ions and ATP depletion induced by pediocin PA-1 are concomitant with cell-death in *Listeria monocytogenes* Scott-A. J Appl Microbiol. 1995; 79 (6): 684-90.

25. Maurya IK, Pathak, S, Sharma M, Sanwa H, Chaudhary P, Tupe S, Deshpande M, Chauhan VS, Prasad R. Antifungal activity of novel synthetic peptides by accumulation of reactive oxygen species (ROS) and disruption of cell wall against Candida albicans. Peptides. 2011; 32 (8): 1732-1740.

26. Sharma A, Srivastava SH. Anti-Candida activity of two-peptide bacteriocins, plantaricins (Pln E/F and J/K) and their mode of action. Fungal Biol. 2014; 118 (2): 264-275.

27. Herranz C, Chen Y, Chung HJ, Cintas LM, Hernandez PE, Montville TJ, Chikindas ML. Enterocin P Selectively Dissipates the Membrane Potential of Enterococcus faecium T136. Appl Environ Microbiol. 2001; 67 (4): 1689-1692.

28. Fujita K, Ichimasa S, Zendo T, Koga S, Yoneyama F, Nakayama J, Sonomoto k. Structural Analysis and Characterization of Lacticin Q, a Novel Bacteriocin Belonging to a New Family of Unmodified Bacteriocins of Gram-Positive Bacteria. Appl Environ Microbiol. 2007; 73 (9): 2871-2877.

29. Gonzalez B, Glaasker E, Kunji ERS, Driessen AJM, Suarez JE. Bactericidal mode of action of plantaricin C. Appl Environ Microbiol. 1996; 62 (8): 2701-2709.

30. Yurong G, Dapeng L, Yan Sh, Xiaoyan L. Mode of action of sakacin C2 against Escherichia coli. Food Control. 2011; 22 (5): 657-661.