P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.038

Anticancer Potential of *Pediococcus pentosaceus* SR6 Protein: Inducing Apoptosis and Necrosis in MCF-7 Breast Cancer Cells

I Wayan Suardana [1,*], Hevi Wihadmadyatami [2] and Dyah Ayu Widiasih [2]

[1]Department of Preventive Veterinary Medicine, Veterinary Public Health Laboratory, The Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia
[2]Department of Anatomy, The Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[3]Depertment of Veterinary Public Health, The Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
***Corresponding author:** wayan_suardana@unud.ac.id

Article History: 25-008 Received: 15-Jan-25 Revised: 16-Mar-25 Accepted: 17-Mar-25 Online First: 12-Apr-25

ABSTRACT

A preliminary study on the anticancer effects of *Pediococcus pentosaceus* SR6 against breast cancer has been carried out. A 28.4kDa protein from this strain was identified as having anticancer properties, as evidenced by morphological changes observed through double staining with Acridine Orange/Propidium Iodide (AO/PI) and DAPI. However, its quantitative efficacy as an anticancer therapy has not yet been investigated. Therefore, this study was conducted to further explore its potential. The study began with the cultivation of *P. pentosaceus* SR6 isolate in a sterile MRS broth medium. Protein fragments of the isolate were then analyzed using the SDS-PAGE method. A specific protein, predicted to have biological activity, was subsequently isolated, purified, and electroeluted before being tested on the MCF-7 breast cancer cell line. Its anticancer potential was evaluated by measuring apoptosis and necrosis activity through flow cytometry. Finally, the data were statistically analyzed using SPSS 25 software. The study results demonstrated that the 28.4kDa protein from *P. pentosaceus* SR6 exhibited cytotoxicity against MCF-7 cells, with an IC_{50} value of 5.42µg/mL. Its anticancer potential was confirmed by a significant reduction in cancer cell viability from 90.67±0.31% to 14.97±0.85%. Flow cytometry analysis revealed an increase in early apoptosis from 3.77±0.06% in the control group to 47.43±0.15% in the treated cells, while late apoptosis rose from 3.03±0.12% to 37.23±1.27%. Similarly, necrotic cell populations increased from 2.70±0.20% to 3.63±0.31%. In the cell cycle analysis, the number of cells in the G1 phase decreased from 47.07±1.15% to 43.40±3.84% following treatment with the 28.4kDa protein. A decline was also observed in the G2-M (division) phase, from 21.83±0.55% to 18.77±1.01%, and in the S (synthesis) phase, from 15.87±0.49% to 13.80±0.17%, corresponding to reductions of 14.05% and 13.03%, respectively. Conversely, the Sub-G1 phase, associated with apoptosis, increased by 4.72%, rising from 3.53±0.21% to 3.70±0.60%. These findings provide quantitative evidence that the 28.4kDa protein from *P. pentosaceus* SR6 exhibits anticancer activity in MCF-7 cells through apoptosis and necrosis. Further research, including preclinical and clinical studies, is necessary before potential therapeutic application.

Key words: Apoptosis, *Pediococcus pentosaceus* SR6, MCF-7 cell line, Vero cells, IC_{50}.

INTRODUCTION

The International Agency for Research on Cancer (IARC), released the latest estimates of the global burden of cancer. The new information available on IARC's Global Cancer Observatory covers 185 countries and 36 cancers showing female breast cancer ranked second (2.3 million cases, 11.6%) under lung cancer in the first position (Anonym 2024). Furthermore, WHO data for 2022 recorded that breast cancer sufferers in Indonesia were at the forefront among other cancers, namely 66,271 cases or 16.2% of the total cases. Breast cancer can occur in both men and women, but the prevalence in women is much higher (Anonym 2021).

In animals, cases of mammary tumors are very rarely reported, and the cases are commonly reported in dogs and cats. Cases of mammary tumors in various animals show variations in type and severity. The researchers found that mammary tumors in eight cat populations of the world proved associated with the Hspb1 mutation (Saif et al. 2016). Fibrosarcoma cases have been reported in the mammary glands of Golden Retriever dogs (Putri and Gorda 2019)

Cite This Article as: Suardana IW, Wihadmadyatami H and Widiasih DA, 2025. Anticancer potential of *Pediococcus pentosaceus* SR6 protein: inducing apoptosis and necrosis in MCF-7 breast cancer cells. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2025.038

and Human basal-like breast cancer is proved to be represented by one of the two mammary tumor subtypes in dogs (Watson et al. 2023). Furthermore, a case of mammary tumor in a 13-year-old tigress was found that had spread to the lungs (Fraser et al. 2024).

Extensive research has been conducted on tumors in both animals and humans. Several studies have contributed to this field, including *A Comparative Approach of Tumor-Associated Inflammation in Mammary Cancer Between Humans and Dogs* (Carvalho et al. 2016), *Neuroendocrine Carcinomas of the Canine Mammary Gland: Histopathological and Immunohistochemical Characteristics* (Nakagaki et al. 2020), and *The Expression Pattern of SMP30 in Mammary Carcinoma in Humans, Dogs, and Cats as a Pan-Species Diagnostic Marker, Facilitating Strategies for Diagnosing Mammary Carcinoma Across Species* (Baek et al. 2021). Additionally, research such as *Rapid Visualization of Mammary Gland Tumor Lesions in Dogs Using the Enzyme-Activated Fluorogenic Probe Gamma-Glutamyl Hydroxymethyl Rhodamine Green* (Hirose et al. 2022) has provided valuable diagnostic insights. A recent study also established a link between breast cancer and the intestinal microbiome in mice as an animal model, with findings that serve as important biomarkers for breast cancer diagnosis (Ji et al. 2023).

Furthermore, the managements of cancer sufferers are chemotherapy, radiotherapy, surgery, hormone therapy, immunotherapy and targeted therapy as the recent method (He and Xu 2021). Principally, cancer therapy is designed to destroy cancer cells. However, due to the lack of specificity in targeting only cancerous cells, this therapy often affects healthy tissues and organs. The general effects of cancer therapy can vary depending on the treatment modality and dosage. The effect commonly involves damage to rapidly dividing normal cells and tissues which leads to various side effects. Several side effects such as bone marrow suppression can lead to conditions like anemia, neutropenia, and thrombocytopenia which increase the risk of infections, fatigue, bleeding (Crawford et al. 2010) and kidney damage (nephrotoxicity) (Chen et al. 2022). This occurs due to drug-induced oxidative stress and apoptosis in renal tubular cells (Ozkok and Edelstein 2014); oral mucosa damage (oral mucositis) that can increases the risk of infections and difficulties in eating and speaking (Villa and Sonis 2015; Al-Dasooqi et al. 2013); inflammation resulting from cancer therapies can activate inflammatory pathways. Cytokines, such as TNF-α, IL-6, and IL-1, are often released, contributing to systemic inflammation, fatigue and tissue damage (Balkwill and Mantovani 2012; Bonavita et al. 2015); disruption of normal metabolism that may lead to hyperglycemia, weight loss, cachexia, or fatigue due to metabolic stress on the body (Blum et al. 2014) and secondary lymphedema resulting from damaged lymphatic vessels. This condition occurs mainly in breast cancer sufferers. This condition causes swelling, pain, and restricted movement in the affected limb (DiSipio et al. 2013).

Ideally, cancer treatment can eliminate tumors and metastases, but they generally still have shortcomings such as requiring additional treatment or being less specific to the target which results in additional side effects (Pucci et al. 2019). Recently, researchers have focused on discovering antitumor agents with minimal side effects, and one such promising candidate is bacteriocins. Bacteriocins is a specific protein with a low molecular weight, produced by lactic acid bacteria (LAB), which have demonstrated potential in targeting various cancer cells (Molujin et al. 2022). The cationic peptides of bacteriocin, synthesized in ribosomes, exhibit selective cytotoxicity against cancer cells while sparing normal cells. This selective mechanism makes bacteriocins a strong candidate for further exploration in cancer therapy and clinical trials (Wu et al. 2021). Mechanisms of bacteriocin as an anticancer are selective membrane disruption. In this action, cancer cell membranes often carry a negative charge due to high levels of anionic phospholipids, distinguishing them from normal cells. Bacteriocins, being cationic, preferentially bind to these negatively charged membranes, leading to membrane destabilization and cell death (Kaur and Kaur 2015). Another mechanism is membrane fluidity. In this theory, the increased fluidity and presence of microvilli in cancer cell membranes facilitate the binding and insertion of bacteriocins, enhancing their cytotoxic effects. (Wang et al. 2024).

The previous study revealed that the LAB strain *Pediococcus pentosaceus* SR6 demonstrated anticancer potential by reducing the viability of T47D cancer cells from 95.08 to 50.65% (Swacita et al. 2022). Furthermore, the next study also proved the anticancer effect of the 28.4kDa protein prom *P. pentosaceus* SR6 in MCF-7 breast cancer cell line qualitatively by double staining with acridine orange/propidium iodide and DAPI staining method. Based on these considerations, the deep study to evaluate the anticancer potential of this strain is quantitatively interesting to present.

MATERIALS AND METHODS

Ethical approval

This Ethical approval was not required in this study because there was no use of live animals.

Cultivation of the SR6 lactic acid bacteria strain isolate

The SR6 lactic acid bacteria strain isolate was retrieved from a 30% glycerol stock that had been stored at -20°C. The isolate was thawed at 4°C for 15min and subsequently cultured at room temperature in a sterile MRS broth medium. It was then incubated at 37°C for 24 hours. Once the isolate had grown, it was prepared for further testing (Suardana et al. 2024; Sukrama et al. 2020).

Protein fragments analysis of lactic acid bacteria SR6 strain

Protein fragments from the isolate were identified and analyzed using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique. The *P. pentosaceus* SR6 culture suspension was centrifuged at 3,000 rpm at 4°C for 10min, washed with 0.1 M PBS buffer (pH 7.0) and centrifuged again under the same conditions. This washing step was repeated three times to ensure purity. Protein extraction was carried out through sonication under cooling conditions, with six cycles of 30-second pulses at 0.7 duty cycle amplitude, interspersed with 5-second intervals. The resulting cell mass was then centrifuged at 13,000rpm for 10min, and the

supernatant was collected as the protein sample for further analysis.

The protein content was quantified using the Bradford assay, and the concentration of the electrophoresed protein samples was standardized through dilution. Protein profiling was performed using SDS-PAGE, employing a 12.5% resolving gel and a 5% stacking gel. A total of 15 µL of protein solution (35 µg/µL) was loaded into each well, except for the first well, which was loaded with a protein marker standard (20–220kDa, Invitrogen LC5602) for molecular weight comparison. Following electrophoresis, the gel was stained with 1% Coomassie Brilliant Blue and subsequently destained using a solution composed of methanol, glacial acetic acid, and distilled water in a 5:1:4 ratio until distinct protein bands were observed. The molecular weights of the protein bands were determined by calculating their retention factors (Rf), comparing the migration distance of each band to that of the standard protein marker using the following formula (Suardana et al. 2013; Suardana et al. 2024):

$$Rf = \frac{\text{The distance of the moving protein band from its starting point}}{\text{The distance of the moving tracker color from its starting place}}$$

Isolation, purification and electroelution of specific proteins

The unstained SDS-PAGE gel was sliced along the specified protein bands. Each gel slice was placed into a cellophane bag and immersed in 1–2mL of 0.2mM phosphate buffer (PB). The bags were then positioned in an electroelution chamber filled with 0.1mM phosphate buffer. Electroelution was carried out in a cooled chamber at 220V and 20mA overnight. To confirm protein elution, the acrylamide gel pieces were stained with Coomassie Blue for 20min. Destaining was performed to check for the absence of bands. If no bands remained, it indicated successful protein elution. The liquid containing the eluted protein from the cellophane bag was then collected, precipitated, and purified with a 1:1 ratio of absolute ethanol. This process yielded protein samples ready for further testing (Suardana et al. 2024).

Culture preparation of MCF-7 cells

The MCF-7 cells used in this study were obtained from the Parasitology Laboratory at Gadjah Mada University. A total of 1mL sample of MCF-7 cell culture, stored in a frozen state in liquid nitrogen (cryotube), was thawed. The cells were washed with DMEM medium by centrifugation at 3,000rpm for 5min at 4°C. The pellet was washed again under the same conditions and then resuspended in 10mL of complete medium, consisting of DMEM supplemented with 10% Newborn Calf Serum (Sigma N4887), 100mg/mL streptomycin, 100IU/mL penicillin, and 50µg/mL fungizone (Fisher Scientific, BW17-745H). The cells were incubated at 37°C in a 5% CO_2 atmosphere. After 24 hours, the growth medium was replaced and the monolayer cell formation was observed over 3–7 days (Liu and Pan 2010; Suardana et al. 2018; Suardana et al. 2024).

Apoptosis and necrosis test

Apoptosis and necrosis tests were performed using flow cytometrically with a double staining method. A 6-well culture plate (Corning) was coated with 50µL of MCF-7 monolayer cells with a cell density of 7 x 10^5cells/well and then incubated overnight at 37°C, with an atmospheric humidity of 5% CO_2. Each well was then added with 10µL of specific protein pellets from electroelution. The cells were then incubated for 24h, the liquid was discarded, and the monolayer cells were washed 2 times with DMEM media. Cells were added with 100µL of complete growth medium, and incubated for 1 day. Cells were harvested using 0.25% trypsin and then washed with PBS. Cells were then treated with 100µL of the Annexin-V-FLUCOS staining kit. Incubation was performed in the dark for 10min at a temperature of 25-27°C. Histograms of apoptotic and necrotic cells were analyzed using a FACSCalybur flow cytometer (Becton-Dickinson). Interpretation of Results; if Annexin V-positive, PI-negative (Q3): Early apoptosis (phosphatidylserine externalization but intact membrane), if Annexin V-positive, PI-positive (Q2): Late apoptosis or secondary necrosis (phosphatidylserine externalization with membrane damage), if Annexin V-negative, PI-positive (Q1): Necrosis (membrane damage without phosphatidylserine externalization), and if Annexin V-negative, PI-negative (Q4): Viable cells (intact membrane and no phosphatidylserine externalization) (Crowley et al. 2016; Vermes et al. 1995).

Data Analysis

Research data were presented descriptively in the form of average and deviation standards. The average value was then analyzed using the General Linear Model and further tested with the Duncan test in the SPSS version 25 program (Santoso 2018).

RESULTS

Protein fragments analysis of lactic acid bacteria SR6

The stock culture of lactic acid bacteria SR6 was propagated in an MRS broth medium. After the bacteria had grown, they were subcultured in a larger volume to prepare for protein analysis. The protein analysis results are presented in Fig. 1.

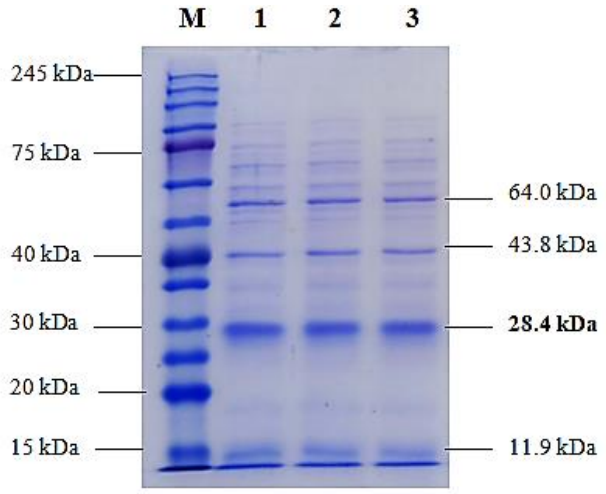


Fig. 1: The SDS-PAGE results of LAB SR6 isolate-protein with 5% stacking gel and 12% resolving gel. M: Marker (PM5100). No: 1- 3 isolate SR6 15µL.

In Fig. 1, there are several protein bands visible, but the one of interest in this study is the protein band with a MW of 28.4kDa of *P. pentosaceus* SR6 because showing thick and distinct bands. The protein bands were then excised and collected to obtain sufficient amounts. The collected proteins were electroeluted and continue to be tested as a treatment on MCF-7 cancer cells based on the IC_{50} previously of 5.42ppm (Suardana et al. 2024).

Apoptosis and necrosis test

The results of the apoptosis and necrosis test of the 28.4kDa protein of *P. pentosaceus* SR6 using the Flowcytometry method based on the IC_{50} as in Fig. 2.

The data in Fig. 2 shows the difference in cell morphology between the control and MCF7 cells that were hybridized with 24.8kDa protein from the *P. pentosaceus* SR6 isolate. The number of living cells in the control was very high with an average of 90.67±0.31%, while in the treatment with 24.8kDa protein, it decreased to 14.97±0.85%. Cells entering the early apoptosis stage for the control were 3.77±0.06% and entering the late apoptosis stage were 3.03+0.12%. On the other hand, the cells that received the treatment increased to 47.43±0.15 and 37.23±1.27%, respectively. Likewise, cells experiencing necrosis increased from 2.70±0.20 to 3.63±0.31%. The research data in Fig. 2 are in line with the research results found by previous study which found that a specific protein of 24.8kDa from the LAB SR6 isolate with Acridine Orange/Propidium Iodide (AO/PI) staining pushed cells into the necrosis phase by only 1.33 and 10% respectively at protein concentrations of 12.5 and 25ppm (Suardana et al. 2022).

Cell cycle test

Cell cycle test is very important to be done as a confirmation of the results of apoptosis and necrosis tests. This test is not only a complement, but also an important step to further understand how an anticancer agent works in inhibiting the growth and development of cancer cells. The results of the cell cycle test from the effect of treatment compared to the control as in Fig. 3.

The cell cycle analysis presented in Fig. 3 demonstrates that MCF-7 cells treated with the 24.8kDa protein from *P. pentosaceus* SR6 exhibited a reduction in the G1 phase (resting phase), decreasing from 47.07±1.15% to 43.40±3.84%, corresponding to a 7.79-fold decline. Similarly, the proportion of cells in the G2-M (division) phase and S (synthesis) phase also decreased following hybridization treatment, from 21.83±0.55% to 18.77±1.01% and from 15.87±0.49 to 13.80±0.17%, representing 14.05-fold and 13.03-fold reductions, respectively. In contrast, the number of cells in the Sub-G1 phase (apoptotic phase) increased 4.72-fold, rising from 3.53±0.21% to 3.70±0.60%.

DISCUSSION

This study demonstrates the anticancer activity of the 28.4kDa protein from *P. pentosaceus* SR6. The protein exhibits cytotoxic effects on MCF-7 cancer cells in a quantitative manner. Its mechanism of action involves inducing apoptosis and necrosis by reducing the proportion of cells in the G1 (resting), G2-M (division) and S (synthesis) phases, while simultaneously increasing the population of cells in the Sub-G1 (apoptotic) phase. The research results obtained also strengthen the evidence of the anticancer effect of the 28.4kDa protein from the LAB SR6 isolate, as well as strengthening the results of previous research which found that the 28.4kDa protein from *P. pentosaceus* SR6 has the potential as an anticancer compound (Suardana et al. 2024). The ability to inhibit cancer growth of the LAB SR6 isolate was in line with the

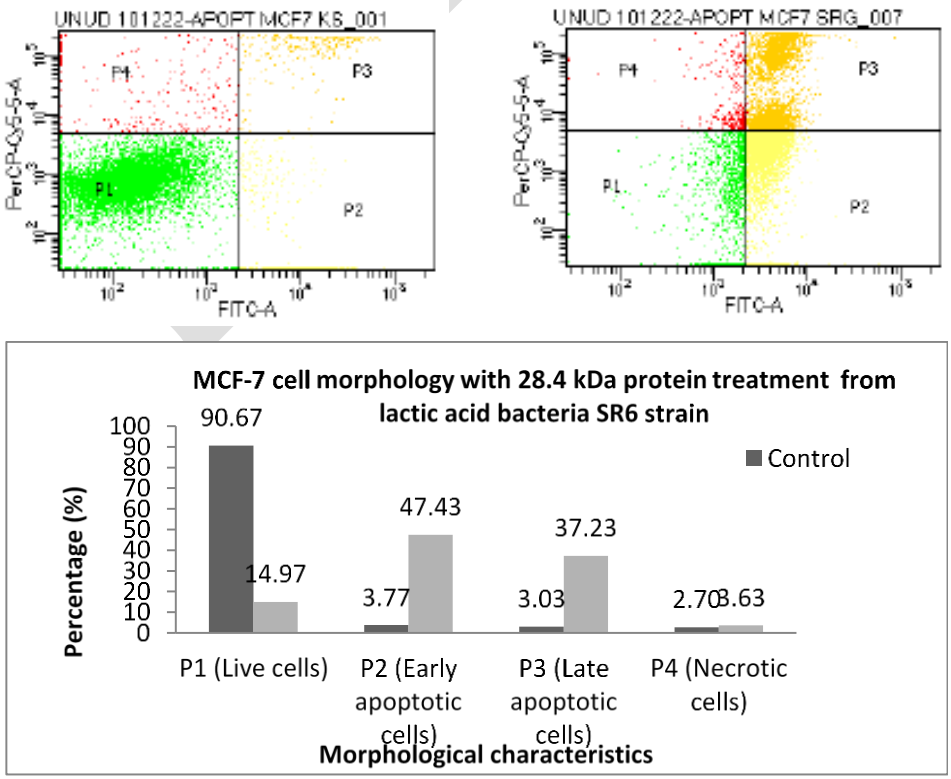
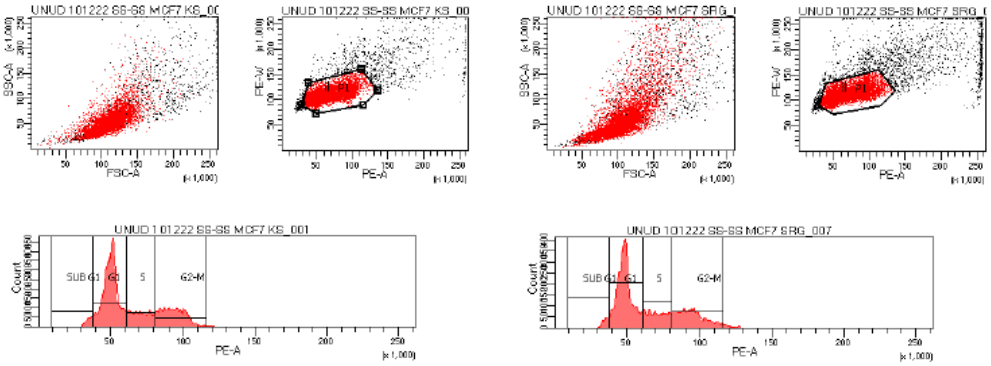


Fig. 2: MCF-7 cell morphology between control and cell treated with 24.8kDa protein hybridization from lactic acid bacteria SR6 strain.



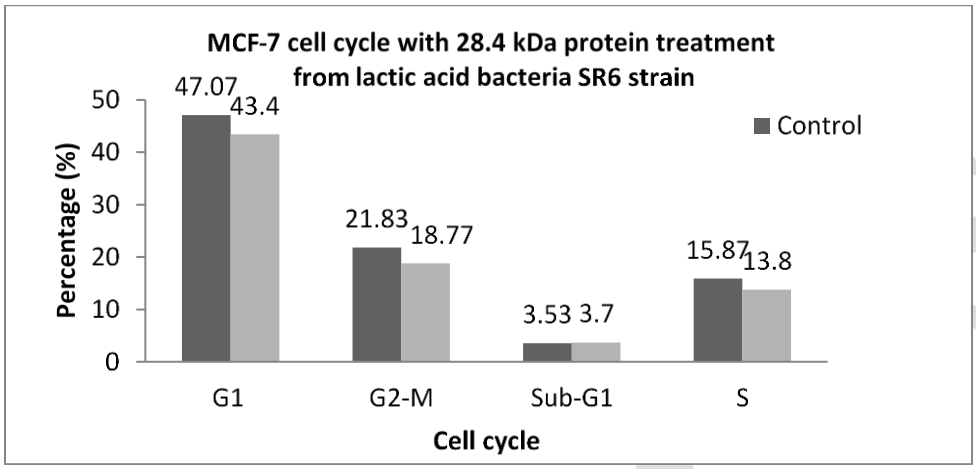


Fig. 3: MCF-7 cell cycle between control and cell treated with 24.8kDa protein hybridization from lactic acid bacteria SR6 strain.

previous founding that proved the anticancer effect of lactic acid bacteria is known through several mechanisms such as antiproliferative activity, encouraging cancer cells to enter the resting phase (cell cycle arrest), antimutagenic mechanisms, antiangiogenic effects, anti-inflammatory effects, and apoptosis induction (Garbacz 2022). The results also proved the previous study, which used nisin as an anticancer, as a bacteriocin produced by Lactococcus lactis. Following treatment with nisin at various doses, cancer cells exhibited an increase in their apoptotic index, the cell cycle stopped, and suppressed the expression of genes that are involved in cell migration and proliferation (Ahmadi et al. 2017).

Bacteriocins promising candidates as anticancer agents, offering potential benefits over conventional therapies. 1) Selective Cytotoxicity: Bacteriocins, particularly cationic and amphiphilic ones, have a high affinity for the negatively charged membranes of cancer cells. This allows them to selectively target and destroy cancer cells without harming healthy cells (Molujin et al. 2022); 2) Induction of Apoptosis: Certain bacteriocins can induce programmed cell death (apoptosis) in cancer cells, leading to their elimination. For instance, the bacteriocin LS10 has been shown to cause apoptotic and necrotic death of cancer cells at varying concentrations (Baindara et al. 2017); 3) Minimal Side Effects: Unlike traditional cancer therapies, bacteriocins have demonstrated the ability to selectively destroy cancer cells without affecting neighboring normal cells, potentially reducing side effects associated with treatment (Wang et al. 2024); 4) Broad-Spectrum Anticancer Activity: Bacteriocins have shown activity against various human cancer cell lines, including breast cancer cells, indicating their potential as versatile anticancer agents (Sharma et al. 2021) and Reduced Risk of Drug Resistance: Bacteriocins can exert rapid tumor-killing effects without the need to interact with specific receptors, decreasing the likelihood of cancer cells developing resistance (Wang et al. 2024).

Bacteriocins, produced by bacteria, have demonstrated potential as anticancer agents by inducing cell death in cancer cells through apoptosis and necrosis pathways. The mechanisms by which bacteriocins exert these effects include: 1) Membrane Disruption: Bacteriocins can interact with cancer cell membranes, leading to depolarization and increased permeability. This disruption can cause cell death through necrosis or initiate apoptotic pathways (Kaur and Kaur 2015); 2) Mitochondrial Pathway Activation: Some bacteriocins increase mitochondrial membrane permeability by altering the Bax/Bcl-2 ratio and promoting reactive oxygen species (ROS) production. This leads to cytochrome c release into the cytoplasm, forming apoptosomes that activate caspase-9, initiating apoptosis (Wang et al. 2024); 3). Death Receptor Pathway Activation: Bacteriocins can promote apoptosis through the death receptor pathway by inhibiting the expression of c-FLIP, an inhibitor of caspase-8. This inhibition allows for the activation of caspase-8, further propagating the apoptotic signal (Wang et al. 2024) and 5). Concentration-Dependent Effects: The effect of bacteriocins on cancer cells can be concentration-dependent. For instance, the bacteriocin LS10 induces apoptotic death at lower concentrations and necrotic death at higher concentrations (Baindara et al. 2017).

Conclusion

Based on these results of the study, the specific protein from the Pediococcus pentosaceus SR6 with a molecular weight (MW) of 28.4kDa is proven to have anticancer

activity in MCF-7 cells through apoptosis and necrosis pathways. Its activity is also strengthened by the results of cell cycle analysis which increases the cell entering the Sub-G1 phase as the apoptosis phase compared with the control. It is needed to continue studies focusing on pre-clinical and clinical studies to complete its effect in vivo before application.

Funding: The publication cost of this article was funded by grant funding.

Acknowledgment: The authors would like to express his gratitude to the Ministry of Agriculture, Republic of Indonesia for providing financial support through the Advanced of Research and Innovation Funding Program at 2nd Stages with the main contract no Letter No. B-3830/II.7.5/FR.06.00/8/2024 dated August 1, 2024.

Conflict of Interest: The authors declare there are no conflicts of interest.

Data Availability: The data is available from the corresponding author(s) based upon a reasonable request.

Authors' Contributions: I Wayan Suardana was responsible for conceptualizing and designing the study, as well as data collection. Hevi Wihadmadyatami contributed to conceptualization, methodology, data curation, resource provision, project administration, and writing review and editing. Dyah Ayu Widiasih supervised the design and formulation of materials in the laboratory and also supervised data. All authors have read, reviewed, and approved the final manuscript.

Generative AI statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Ahmadi S, Ghollasi M and Hosseini HM, 2017. The apoptotic impact of nisin as a potent bacteriocin on the colon cancer cells. Microbial Pathogenesis 111: 193-197 https://doi.org/10.1016/j.micpath.2017.08.037

Al-Dasooqi N, Sonis ST, Bowen JM, Bateman E, Blijlevens N, Gibson RJ, Logan RM, Nair RG, Stringer AM, Yazbeck R, Elad S, Lalla RV and Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral O, 2013. Emerging evidence on the pathobiology of mucositis. Support Care Cancer 21(11): 3233-3241. https://doi.org/10.1007/s00520-013-1900-x

Anonym, 2021. World health organization (who). 10 types of cancer with the highest number of national cases.

Anonym, 2024. Global cancer burden growing, amidst mounting need for services. 1 February 2024 ed.,

Baek SM, Lee SW, Kim TU, Choi SK, Yun S, Lee WJ, Han SH, Hong IH, Park SJ, Kim TH, Jeong KS and Park JK, 2021. Senescence marker protein 30 (smp30): A novel pan-species diagnostic marker for the histopathological diagnosis of breast cancer in humans and animals. International Journal of Molecular Sciences 22(5): 2340. https://doi.org/10.3390/ijms22052340

Baindara P, Gautam A, Raghava GPS and Korpole S, 2017. Anticancer properties of a defensin like class iid bacteriocin laterosporulin10. Scientific Reports 7: 46541.https://doi.org/10.1038/srep46541

Balkwill FR and Mantovani A, 2012. Cancer-related inflammation: Common themes and therapeutic opportunities. Seminars in Cancer Biology 22(1): 33-40. https://doi.org/10.1016/j.semcancer.2011.12.005

Blum D, Stene GB, Solheim TS, Fayers P, Hjermstad MJ, Baracos VE, Fearon K, Strasser F, Kaasa S and Euro I, 2014. Validation of the consensus-definition for cancer cachexia and evaluation of a classification model--a study based on data from an international multicentre project (epcrc-csa). Annals of Oncology 25(8): 1635-1642. https://doi.org/10.1093/annonc/mdu086

Bonavita E, Galdiero MR, Jaillon S and Mantovani A, 2015. Phagocytes as corrupted policemen in cancer-related inflammation. Advances in Cancer Research 128: 141-171. https://doi.org/10.1016/bs.acr.2015.04.013

Carvalho MI, Silva-Carvalho R, Pires I, Prada J, Bianchini R, Jensen-Jarolim E and Queiroga FL, 2016. A comparative approach of tumor-associated inflammation in mammary cancer between humans and dogs. Biomed Research International 2016: 4917387. https://doi.org/10.1155/2016/4917387

Chen C, Xie D, Gewirtz DA and Li N, 2022. Nephrotoxicity in cancer treatment: An update. Advances in Cancer Research 155: 77-129. https://doi.org/10.1016/bs.acr.2022.03.005

Crawford J, Caserta C, Roila F and Group EGW, 2010. Hematopoietic growth factors: Esmo clinical practice guidelines for the applications. Annals of Oncology 21(Su5): v248-251. https://doi.org/10.1093/annonc/mdq195

Crowley LC, Marfell BJ, Scott AP and Waterhouse NJ, 2016. Quantitation of apoptosis and necrosis by annexin v binding, propidium iodide uptake and flow cytometry. Cold Spring Harb Protoc 2016(11): https://doi.org.10.1101/pdb.prot087288

DiSipio T, Rye S, Newman B and Hayes S, 2013. Incidence of unilateral arm lymphoedema after breast cancer: A systematic review and meta-analysis. The Lancet Oncology 14(6): 500-515. https://doi.org/10.1016/S1470-2045(13)70076-7

Fraser C, Keong Kok M, Shameha Abdul Razak I, Puspitasari Y and Salleh A, 2024. Diagnostic challenge in veterinary pathology: Metastatic mammary tumor in a female tiger (panthera tigris). Veterinary Pathology 61(4): 508-511. https://doi.org/10.1177/03009858241226650

Garbacz K, 2022. Anticancer activity of lactic acid bacteria. Seminars in cancer biology 86(Pt 3): 356-366. https://doi.10.1016/j.semcancer.2021.12.013

He Z and Xu JG, 2021. Evaluation of the efficiency and safety of combined chemotherapy and molecular-targeted therapy in the treatment of advanced gastric cancer: A protocol for systematic review and meta-analysis. Medicine (Baltimore) 100(45): e27557. https://doi.org/10.1097/MD.0000000000027557

Hirose Y, Uchida M, Tsuboi M, Nakagawa T, Yaga L, Maeda S, Momoi Y, Kuriki Y, Kamiya M, Urano Y and Yonezawa T, 2022. Rapid visualization of mammary gland tumor lesions of dogs using the enzyme-activated fluorogenic probe; gamma-glutamyl hydroxymethyl rhodamine green. The Journal of Veterinary Medical Science 84(4): 593-599. https://doi.org/10.1292/jvms.22-0003

Ji X, Li R, Hu X, Tian Y, Liu L, Zhang C, Xu L, Chen Y, Xie H, Mao L, Cai T and Li W, 2023. Construction of model animals to explore intestinal microbiome for detection of breast cancer. PLoS One 18(5): e0280971. https://doi.org/10.1371/journal.pone.0280971

Kaur S and Kaur S, 2015. Bacteriocins as potential anticancer agents. Frontiers in Pharmacology 6: 272. https://doi.org/10.3389/fphar.2015.00272

Liu CF and Pan TM, 2010. In vitro effects of lactic acid bacteria on cancer cell viability and antioxidant activity. Journal of Food and Drug Analysis 18(2): 77-86. https://doi.org/10.38212/2224-6614.2287

Molujin AM, Abbasiliasi S, Nurdin A, Lee PC, Gansau JA and Jawan R, 2022. Bacteriocins as potential therapeutic approaches in the treatment of various cancers: A review of in vitro studies. Cancers (Basel) 14(19). https://doi.org/10.3390/cancers14194758

Nakagaki KYR, Nunes MM, Garcia APV, De Brot M and Cassali GD, 2020. Neuroendocrine carcinomas of the canine mammary gland: Histopathological and immunohistochemical characteristics. Frontiers in Veterinary Science 7: 621714. https://doi.org/10.3389/fvets.2020.621714

Ozkok A and Edelstein CL, 2014. Pathophysiology of cisplatin-induced acute kidney injury. Biomed Research International 2014: 967826.https://doi.org/10.1155/2014/967826

Pucci C, Martinelli C and Ciofani G, 2019. Innovative approaches for cancer treatment: Current perspectives and new challenges. Ecancer Medical Science 13: 961. https://doi.org/10.3332/ecancer.2019.961

Putri RK and Gorda IW, 2019. Case study: Fibrosarcoma mammary gland in golden retriever dog. Indonesia Medicus Veterinus 8(3): 404-413. https://doi.org/10.19087/imv.2019.8.3.404

Saif R, Awan AR, Lyons L, Gandolfi B, Tayyab M, Ellahi Babar M and Wasim M, 2016. Mammary tumor associated hspb1 mutation and screening of eight cat populations of the world. Iranian Journal of Veterinary Research 17(4): 231-236.

Santoso S, 2018. *Mastering statistic with spss 25*: PT. Elex Media Komputindo.

Sharma P, Kaur S, Chadha BS, Kaur R, Kaur M and Kaur S, 2021. Anticancer and antimicrobial potential of enterocin 12a from enterococcus faecium. BMC Microbiology 21(1): 39. https://doi.org/10.1186/s12866-021-02086-5

Suardana IW, Pinatih KJP, Widiasih DA, Artama WT, Asmara W and Daryono BS, 2018. Regulatory elements of stx2 gene and the expression level of shiga-like toxin 2 in escherichia coli o157:H7. Journal of Microbiology Immunology and Infection 51(1): 132-140. https://doi.org/10.1016/j.jmii.2016.04.006

Suardana IW, Putra Pinatih KJ, Ayu Ratnawati NLK and Widiasih DA, 2013. Protein profile analysis of escherichia coli o157:H7 from human and animals origin. International Journal of Current Microbiology and Applied Sciences 2(6): 1-11.

Suardana IW, Swacita IBN, Pinatih KJP, Suharsono H and Widiasih DA, 2022. Assessment of cell cycle and induction of apoptosis by shiga-like toxin produced by escherichia coli o157:H7 in t47d breast cancer cells using flow cytometry. Asian Pacific Journal of Cancer Prevention 23(10): 3247-3252. https://doi.org/10.31557/APJCP.2022.23.10.3247

Suardana IW, Wihadmadyatami H and Widiasih DA, 2024. Anticancer activity of the 28.4kDa protein from *Pediococcus pentosaceus* sr6 in mcf-7 breast cancer cell line. International Journal of Veterinary Science 13(6): 724-728. https://doi.org/10.47278/journal.ijvs/2024.158

Sukrama IDM, Franciska J and Suardana IW, 2020. Evaluation of the bacteriocin produced by strain 9 lactic acid bacteria isolate for biopreservation. Veterinary World 13(9): 2012-2019. https://doi.org/10.14202/vetworld.2020.2012-2019

Swacita IBN, Suardana IW, Sudisma IGN and Wihadmadyatami H, 2022. Molecular identification of lactic acid bacteria sr6 strain and evaluation of its activity as an anticancer in t47d cell line. Veterinary World 15(6): 1583-1588. https://doi.org/10.14202/vetworld.2022.1583-1588

Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C, 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled annexin v. Journal of Immunological Methods 184(1): 39-51. https://doi.org/10.1016/0022-1759(95)00072-i

Villa A and Sonis ST, 2015. Mucositis: Pathobiology and management. Current Opinion in Oncology 27(3): 159-164. https://doi.org/10.1097/CCO.0000000000000180

Wang Y, Wang Y, Sun T and Xu J, 2024. Bacteriocins in cancer treatment: Mechanisms and clinical potentials. Biomolecules 14(7). https://doi.org/10.3390/biom14070831

Watson J, Wang T, Ho KL, Feng Y, Mahawan T, Dobbin KK and Zhao S, 2023. Human basal-like breast cancer is represented by one of the two mammary tumor subtypes in dogs. Breast Cancer Research 25(1): 114. https://doi.org/10.1186/s13058-023-01705-5

Wu J, Zhang Y, Ye L and Wang C, 2021. The anti-cancer effects and mechanisms of lactic acid bacteria exopolysaccharides in vitro: A review. Carbohydrate Polymers 253: 117308. https://doi.org/10.1016/j.carbpol.2020.117308