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Evaluating the Cytotoxic Activity of *Lactobacillus plantarum* IIA-1A5 against MCF-7 Human Breast Cancer Cells and Identifying Its Surface Layer Protein Gene

(Menilai Aktiviti Sitotoksik *Lactobacillus plantarum* IIA-1A5 terhadap Sel Kanser Payudara Manusia MCF-7 dan Mengenal Pasti Gen Protein Lapisan Permukaannya)

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ABSTRACT

Breast cancer is a serious global health concern, with a high mortality rate worldwide. Using natural compounds as potential cancer therapies is a promising approach to address this issue. Previous research has shown that probiotic lactic acid bacteria (LAB) and their metabolites, such as surface layer protein (*slp*), have a positive impact on a variety of health disorders, including cancer. The purpose of this study was to evaluate the ability of *Lactobacillus plantarum* IIA-1A5 to suppress the growth of the MCF-7 breast cancer cell line and detect the presence of the *slp* gene. Intracellular and extracellular protein fractions were isolated from *L. plantarum* IIA-1A5 cultures. The protein concentrations and molecular weights of the extracts were measured. The anticancer activity of the extracts was assessed using the MTT cytotoxicity test, and IC_{50} values were calculated. The *slp* gene was identified through polymerase chain reaction (PCR) amplification and nucleotide sequencing. The results demonstrated that *L. plantarum* IIA-1A5 had a concentration-dependent inhibitory effect on MCF-7 breast cancer cells, with IC_{50} values of 6.831 and 12.35 µg/mL for intracellular extracts and extracellular extracts, respectively. Additionally, PCR amplification and nucleotide sequencing confirmed the presence of the *slp* gene, which may contribute to the strain's anticancer abilities. These findings suggest the potential of *L. plantarum* IIA-1A5 as a natural anticancer agent against MCF-7 breast cancer cells. Further research is warranted to elucidate the underlying mechanisms of *L. plantarum* IIA-1A5 in breast cancer treatment.

Keywords: Anticancer; breast cancer; lactic acid bacteria; MCF-7; *slp* gene

ABSTRAK

Kanser payudara merupakan isu kesihatan global utama dengan kadar kematian yang tinggi di seluruh dunia. Penggunaan sebatian semula jadi sebagai terapi kanser merupakan strategi yang berpotensi untuk menangani isu ini. Kajian terdahulu telah mencadangkan bahawa bakteria asid laktik probiotik (LAB) dan metabolitnya seperti protein lapisan permukaan (*slp*) telah menunjukkan kesan yang baik terhadap pelbagai keadaan kesihatan, termasuk kanser. Kajian ini bertujuan untuk menyelidik potensi *Lactobacillus plantarum* IIA-1A5 dalam menghalang pertumbuhan sel kanser payudara MCF-7 dan mengenal pasti kehadiran gen *slp*. Fraksi protein intrasel dan ekstrasel diekstrak daripada kultur *L. plantarum* IIA-1A5. Konsentrasi protein dan berat molekul ekstrak dianalisis. Aktiviti antikanser ekstrak dinilai menggunakan ujian sitotoksik MTT dan nilai IC_{50} ditentukan. Amplifikasi rantai polimerase (PCR) dan penjujukan nukleotida dilakukan untuk mengenal pasti gen *slp*. Hasil kajian menunjukkan bahawa *L. plantarum* IIA-1A5 menunjukkan kesan perencatan yang bergantung kepada kepekatan ke atas sel kanser payudara MCF-7, dengan nilai IC_{50} 6.831 µg/mL untuk ekstrak intrasel dan 12.35 µg/mL untuk ekstrak ekstrasel. Kehadiran gen *slp* disahkan dalam strain tersebut melalui amplifikasi PCR dan penjujukan nukleotida. Penemuan ini menunjukkan potensi *L. plantarum* IIA-1A5 sebagai agen antikanser semula jadi untuk rawatan kanser payudara MCF-7. Gen *slp* yang dikenal pasti mungkin menyumbang kepada aktiviti antikanser strain tersebut. Penyelidikan lanjut diperlukan untuk memahami mekanisme asas *L. plantarum* IIA-1A5 dalam rawatan kanser payudara.

Kata kunci: Antikanser; bakteria asid laktik; gen *slp*; kanser payudara; MCF-7

Introduction

Breast cancer is the most commonly diagnosed cancer worldwide, with an estimated 2.3 million new cases in 2020 (Sung et al. 2021). According to Harbeck et al. (2019), breast cancer is the leading cause of cancer-related deaths in women globally. There are a variety of treatment methods for breast cancer, including surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy (Waks & Winer 2019). However, there are major challenges to breast cancer treatment. Chemoresistance is one such challenge, and it significantly diminishes the effectiveness of chemotherapy (Cao et al. 2021; Luqmani 2005). In addition, chemotherapy could potentially disrupt normal metabolic processes (Dennert & Horneber 2006). To overcome these drawbacks, researchers have been exploring the use of natural products as potential treatments for breast cancer.

Probiotic lactic acid bacteria (LAB) and their metabolites have garnered significant attention for their potential health benefits. Their ability to effectively enhance the immune response, improve gastrointestinal function, bolster antioxidant activities, reduce blood glucose concentration, and lower cholesterol levels has been well-documented (Gupta, Jeevaratnam & Fatima 2018; Nowroozi, Mirzaii & Norouzi 2004). These compelling effects suggest that LAB may have a positive impact on overall health and potentially prevent and manage specific health conditions (Mathur, Beresford & Cotter 2020; Nowak, Paliwoda & Blasiak 2019). Furthermore, previous research findings have confirmed the anticancer potential of LAB. LAB-based probiotics and their metabolites have exhibited properties that can impede or suppress the growth of cancer cells, as reported in previous studies (Ding et al. 2018; Nowak, Paliwoda & Blasiak 2019). Liu et al. (2021) discovered that the anti-cancer properties of LAB can be attributed to their active substances, which can be categorized into five main groups: extracellular polysaccharides (EPS), peptidoglycan, nucleic acid, bacteriocin, and S-layer proteins (*slp*). These bioactive compounds play a significant role in the ability of LAB to inhibit proliferating cancer cells.

Lactobacillus plantarum IIA-1A5 is an indigenous LAB isolated from Indonesian beef. Based on previous studies, this compound exhibits probiotic properties (Arief et al. 2015, 2013). Previously, *in vitro* studies demonstrated that protein extracts derived from *L. plantarum* IIA-1A5 exhibited remarkable inhibitory activity against HeLa cervical cancer cells (Ningtiyas et al. 2021) and WiDr colon cancer cells (Adiyoga et al. 2022). However, this strain's ability to inhibit breast cancer cells has yet to be investigated. It is important to note that the spectrum of anticancer activity among LAB strains can vary significantly (Liu et al. 2021). Hence, further investigation of the *L. plantarum* 1A5 strain's anticancer activity against a broader range of cancer cell types will enable us to determine the full extent of its efficacy. Moreover, while the protein extract derived from this strain has shown remarkable inhibitory activity, the specific proteins within the extract that contribute to this activity are yet to be identified. Liu et al. (2021) previously suggested that the S-layer protein (*slp*) of LAB could be the substance responsible for the observed anticancer effects. However, it remains uncertain whether the *L. plantarum* IIA-1A5 strain harbors the gene encoding the *slp* protein.

This current study extends the investigation of the anticancer activity of the protein extract from the *L. plantarum* 1A5 strain against MCF-7 breast cancer cells. It also represents the first attempt to identify the presence of the *slp* gene within its genome. To the best of our knowledge, this is the first report documenting the activity of the *L. plantarum* strain against MCF-7 cells and the potential presence of the *slp* gene.

Materials and Methods

Culture preparation

L. plantarum IIA-1A5 culture was obtained from the Laboratory of Animal Product Technology at IPB University in Bogor, Indonesia. Culture propagation was done based on the method described by Arief et al. (2015). Briefly, 1 mL stock culture was inoculated into 9 mL de Man, Rogosa, and Sharpe (MRS) broth and incubated for 24 h at 37 °C. This process was performed in triplicate to obtain three final cultures used in further analysis.

Extraction of intracellular protein fraction

The intracellular protein fraction was extracted using a modified method by Adiyoga et al. (2022). Approximately 100 mL of *L. plantarum* IIA-1A5 culture was centrifuged at 8,000 × g at 4 °C for 15 min to obtain the pellet containing bacterial cells. Then, the pellet was suspended in 10 mL PBS solution, and 500 µL of lysozyme (40 mg/mL) was added followed by incubation at 37 °C for 1 h. The suspension was subjected to 5 cycles of sonication (2

min run, 1 min rest), and then centrifuged at 10,000 × g at 4 °C for 20 min to obtain the supernatant. The collected supernatant is the intracellular extract.

EXTRACTION OF EXTRACELLULAR PROTEIN FRACTION

The extracellular protein fraction was extracted using a modified method by Adiyoga et al. (2022). Approximately 100 mL of *L. plantarum* IIA-1A5 culture was centrifuged at 8,000 × g at 4 °C for 15 min to obtain the cell-free supernatant (CFS). The CFS was filtered using a Minisart 0.22 µm membrane filter (Sartorius AG, Germany). The dialysis process was performed in 0.02 M potassium phosphate buffer (pH 6.2) using a Wako dialysis membrane size 20 (FUJIFILM Wako Chemicals, Japan). The end product of the dialysis process is the extracellular extract.

DETERMINATION OF PROTEIN CONCENTRATION AND MOLECULAR WEIGHT

Bradford's protein assay was used to determine the protein concentration in both intracellular and extracellular protein extracts (Bradford 1976). Bovine serum albumin (BSA) (Sigma-Aldrich, USA) was used as the standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the protein's molecular weight (Laemmli 1970). Acrylamide gel was prepared in the form of resolving gel 12.5% and stacking gel 4%. Next, electrophoresis was conducted at 60V using a Bio-Rad electrophoresis cell (Bio-Rad, USA) with PM2700 ExcelBand™ 3-color Broad Range Protein Marker (Smobio, Taiwan) as a protein ladder. The size of each protein band from the gel was calculated by using the method recommended by Sahlan et al. (2018).

ANALYSIS OF ANTICANCER ACTIVITY

The anticancer activity was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Ningtiyas et al. 2021) on MCF-7 breast cancer cell line. Treatments were prepared using intracellular and extracellular extracts with final protein concentrations of 0, 15, 50, 100, and 200 µg/mL. Doxorubicin was used as a positive control. Based on the MTT assay results, the IC_{50} values were determined using GraphPad Prism 8 software (GraphPad Software Inc., USA).

EXTRACTION OF GENOMIC DNA AND *slp* GENE AMPLIFICATION

Bacterial cultures of *Lactobacillus plantarum* IIA-1A5were harvested at an optical density of 600 nm (OD600) of 6.9. Genome extraction was performed using a DNeasy Plant Mini Kit (Qiagen, Germany). The extracted genomic DNA was then analyzed for its quality and quantity using 1% agarose gel electrophoresis and NanoDrop One (Thermo Fisher Scientific, USA), respectively.

To identify the presence of the *slp* gene in the genomic DNA of the *L. plantarum* 1A5 strain, we performed the polymerase chain reaction (PCR) to amplify the *slp* gene. The degenerate primers used in this study were designed from the alignment of various known *Lactobacillus slp* gene sequences deposited in the Gene Bank. Three reverse primers (R1, R2, and R3) and one forward primer (F4) were obtained (Table 1). The primers used for the amplification were paired as follows: R1-F4, R2-F4, and R3-F4. The amplification was performed using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA) under an Applied Biosystems™ 2720 thermal cycler (Thermo Fisher Scientific, USA) following the parameters shown in Table 2.

TABLE 1. Oligonucleotides used as primers in PCR reactions

Oligonucleotides	Nucleotide sequence (5'→3')
R1	CGYAACACBVAGTTWCATGC
R2	CATWTGYATHCGYAACACBVAGTT
R3	ATCATDMVRAMYGGYAAGAAC
F4	ATGAAGAAAAATTTAAGAA

TABLE 2. PCR setting

PCR setting	Temperature (°C)	Time
Pre-denaturation	95	2 min
Denaturation	85	30 s
Annealing	36 or 41	30 s
Extension	72	2 min
Final extension	72	7 min
Hold	4	∞

DNA SEQUENCE ANALYSIS

The amplicon obtained from PCR was excised from the agarose gel and purified using the Gel/PCR DNA Isolation System (Viogene BioTek, Taiwan). The amplicon was then inserted into a pGEM®-T Easy Vector (Promega, USA). It was used to transform *Escherichia coli* DH5α competent cells using the heat-shock technique spread on 2xYT agar plates containing 35 µg/mL ampicillin, X-Gal (5-Bromo-4-Chloro-3-Indolyl β-D-Galactoside), and IPTG (isopropyl β-D-1-thiogalactopyranoside). The competent cells were incubated at 37 °C overnight, and the white *E. coli* colonies were selected for the plasmid extraction using the alkaline lysis method (Birnboim & Doly 1979). The presence of the inserted PCR product in the purified plasmids was evaluated through a double digest restriction check using ApaI and NdeI restriction enzymes (NEB, USA).

The plasmids were analyzed using Sanger sequencing (Genewiz, Japan) with universal M13 primers as sequencing primers. The DNA sequence was processed using MEGA 11. Next, the processed sequences were analyzed using NCBI BLAST by comparing all combinations of nucleotide or protein queries within the NCBI database. Multiple sequences analysis (MSA) was conducted using Clustal Omega (EMBL-EBI, UK).

STATISTICAL ANALYSIS

Statistical analysis of data was carried out using Minitab 20 (Minitab, LLC), and the results data were expressed as mean ± standard deviation. The data from the anticancer analysis were analyzed using a two-way ANOVA followed by Tukey's posthoc test to assess the differences among means. A p-value < 0.05 indicates that the data is statistically significant (Steel, Torrie & Dickey 1997).

RESULTS AND DISCUSSION

PROTEIN CONCENTRATION AND PROFILES

The results in Table 3 demonstrate that the intracellular extract had a higher protein concentration than the extracellular extract, which is in line with the previous findings of Ningtiyas et al. (2021). Furthermore, previous studies by Nogueira, Touchon and Rocha (2012) have indicated that bacteria typically secrete approximately 5-6% of their total proteins into the extracellular environment, suggesting that intracellular proteins tend to be more abundant than extracellular fractions. Based on these findings, extracellular protein concentrations are typically lower compared to intracellular proteins. In the context of cancer inhibition properties, it is postulated that the protein compounds present in the extract act as bioactive components and are responsible for the observed anticancer effects. Liu et al. (2021) reported two specific proteins found in LAB that have been associated with anticancer properties. These proteins are the S-layer protein or *slp* (Zhang et al. 2020) and bacteriocin (Paiva et al. 2012). It should be noted that while bacteriocin is excreted outside the cells (extracellular), the *slp* is localized on the cell surface and is often associated with sugar moieties (Ford, Normellini & Smit 2007).

Figure 1 displays the SDS-PAGE analysis, which showed the presence of a protein band with an apparent size of approximately 42 kDa in the intracellular fraction; this band was absent in the extracellular fraction. Both fractions also exhibited bands below 10 kDa (Figure 1). However, the <10 kDa protein bands in the intracellular region were significantly thicker compared to those in

the extracellular region. This observation indicates that the concentration of these proteins in the intracellular fraction surpasses that in the extracellular fraction.

Although the identity of each protein band remains unknown, the SDS-PAGE results corroborate the higher protein content in the intracellular fraction compared to the extracellular fraction. Moreover, the exclusive presence of the 42 kDa protein band in the intracellular fraction suggests its unique ability in this compartment. Interestingly, the S-layer protein (*slp*) of *L. acidophilus* ATCC 700396 is approximately 43 kDa in size (Boot et al. 1993), while other *Bacillus* species have *slp* proteins ranging in size from 25 to 71 kDa (Chen et al. 2007). Hence, it is plausible to speculate that the 42 kDa protein band corresponds to the *slp* protein of the *L. plantarum* IIA-1A5 strain. However, the thickness of the protein band in SDS-PAGE is atypical for the *slp* gene. The expression level of this protein is relatively low and typically requires sensitive SDS-PAGE or antibody-based detection methods.

ANALYSIS OF ANTICANCER ACTIVITY

MTT cytotoxicity assays were performed to evaluate the anticancer activity of the samples. The results shown in Table 4 indicate that all extracts displayed concentration-dependent inhibitory activity against MCF-7 cells. This suggests that higher concentrations of the extracts corresponded to increased inhibition of cell growth. Notably, the intracellular fraction of *L. plantarum* IIA-1A5 exhibited superior overall anticancer activity.

TABLE 3. Protein concentration of *L. plantarum* extracts

Extract type	Protein concentration (mg/mL)
Intracellular	0.446 ± 0.07
Extracellular	0.363 ± 0.01

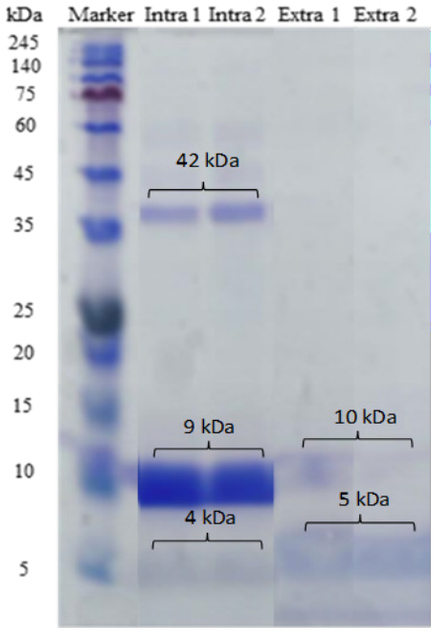


FIGURE 1. SDS-PAGE result

Furthermore, the anticancer activity of the intracellular extracts was comparable to that of doxorubicin, a chemotherapy drug. The intracellular fraction achieved the highest cancer cell growth inhibition of 98.34% ± 0.58, highlighting its considerable potential as an anticancer agent.

The viability of MCF-7 cells was further confirmed through visual examination (Figure 2). Microscopic observations showed that adding probiotic extracts led to cell death (Figure 2(C)-2(K)). In contrast, normal MCF-7 cells were noted in the negative control group, which did not receive any treatment (Figure 2(B)). Therefore, the exposure of LAB extracts to MCF-7 cells led to cell shrinkage resulting in cell death. The metabolite compounds derived from LAB have been implicated in causing cancer cell death through various mechanisms such as inducing apoptosis and necrosis, anti-proliferation, inhibiting the cell cycle, and suppression of angiogenesis (Ren et al. 2003).

The IC_{50} values were determined by analyzing the absorbance-concentration curve (Figure 3). These values indicate the concentration at which a specific biological process is inhibited by 50%, thus indicating the potency of an antagonist drug in pharmacological research (Aykul & Martinez-Hackert 2016). A low IC_{50} value shows that the extracts are effective at inhibiting cancer growth at low concentrations. The results showed that the IC_{50} value of the intracellular extracts (6.831 µg/mL) was significantly lower than that of the extracellular extracts (12.35 µg/mL). This finding suggests that the intracellular extract exhibits higher cytotoxic activity and is better able to inhibit the growth of MCF-7 cells compared to the extracellular extract. As outlined by the United States National Cancer Institute, a crude extract is generally recognized to exhibit *in vitro* cytotoxicity if the IC_{50} falls below 30 µg/mL (Oskoueian et al. 2011). Based on the classification of antiproliferative activities by IC_{50} values (Atjanasuppat et al. 2009), both the intracellular and extracellular extracts can be categorized as active cytotoxic agents against cancer cells ($IC_{50} \leq 20$ µg/mL).

The inhibitory effect of the *L. plantarum* IIA-1A5 extract on breast cancer cells aligns with findings from previous studies that have investigated the anticancer activity of other LABs. For instance, the supernatants of *Bacillus coagulans* (GBI-30, 6068) showed a significant cytotoxic effect on the MCF7 cell line by inducing apoptosis in cancer cells through the mitochondrial pathways, with reduced cytotoxicity on the normal cell line (Dolati et al. 2021). In addition, human breast adenocarcinoma cells (MDA-MB-231) were treated with heat-killed cells and the cytoplasmic fractions of local lactobacilli strains (*L. paracasei* ssp. *paracasei* NTU 101 and *L. plantarum* NTU 102) isolated in Taiwan. They showed the highest percentage of arrested at G0/G1 phase in the cell cycle, thus indicating potent anticancer activity (Liu & Pan 2010). Meanwhile, Aragón et al. (2015) reported that milk fermented by *L. casei* CRL 431 was able to decrease tumor growth in a murine breast cancer model. This was achieved by modulating the immune system and reducing the infiltration of macrophages in the tumor, as well as increasing the level of CD4+ and CD8+ T cells in the mammary glands. The results suggest that LAB extracts, including both extracellular and intracellular fractions, have significant potential as natural alternatives for inhibiting the growth of breast cancer cells. In addition, these findings provide support for further exploration and research into using LAB extracts as potential agents for breast cancer treatment.

TABLE 4. MCF-7 cells growth inhibition (%)

Sample	Dose (µg/mL)				
	0	15	50	100	200
Intracellular	0[c]	94.69 ± 0.52[a]	98.22 ± 0.90[a]	98.34 ± 0.58[a]	98.00 ± 0.53[a]
Extracellular	0[c]	59.79 ± 6.12[b]	94.18 ± 0.64[a]	97.07 ± 2.58[a]	98.00 ± 0.08[a]
Doxorubicin	0[c]	93.75 ± 1.12[a]	94.27 ± 0.84[a]	95.44 ± 1.53[a]	93.80 ± 0.49[a]

[a] Different superscript in the same column indicate significant differences ($p<0.05$)

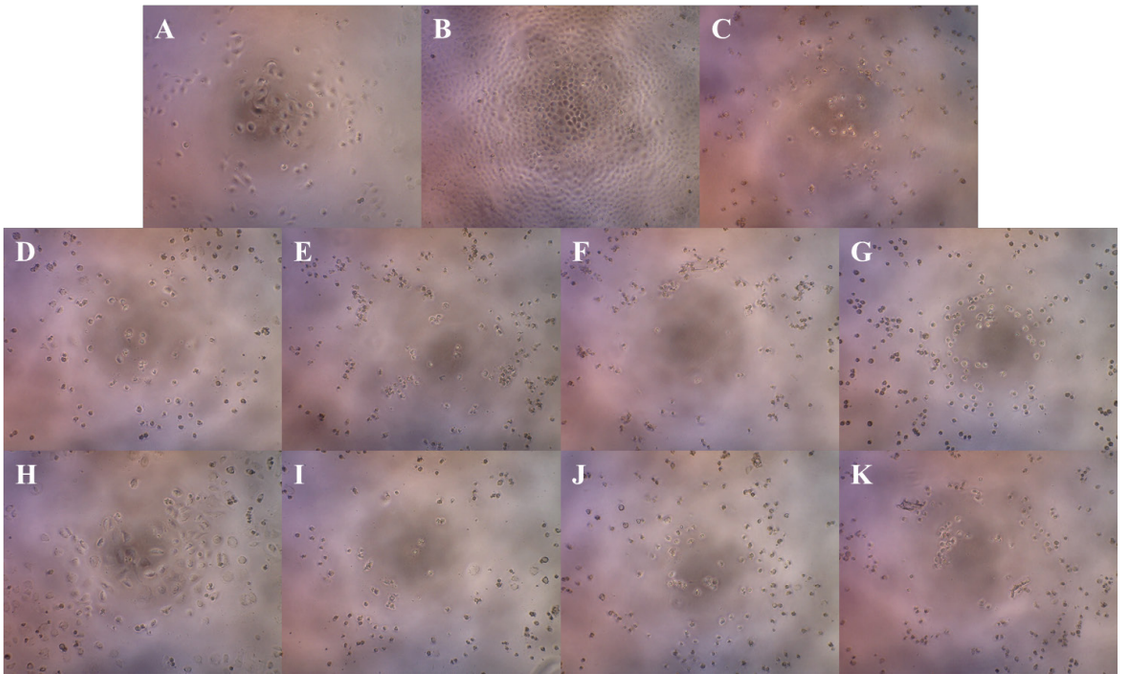


FIGURE 2. MCF-7 cell morphology in different states. A: before treatment; B: negative control; C: doxorubicin 15 μg/mL; D: intracellular 15 μg/mL; E: intracellular 50 μg/mL; F: intracellular 100 μg/mL; G: intracellular 200 μg/mL; H: extracellular 15 μg/mL; I: extracellular 50 μg/mL; J: extracellular 100 μg/mL; K: extracellular 200 μg/mL

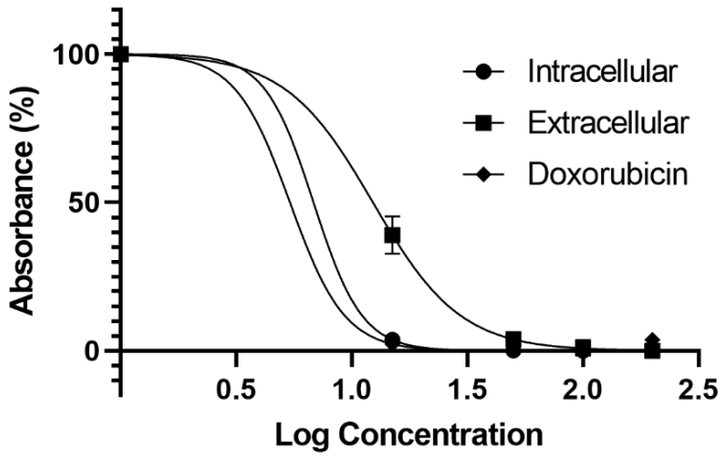


FIGURE 3. IC_{50} value of *L. plantarum* IIA-1A5 protein fraction extracts

IDENTIFICATION OF SURFACE LAYER PROTEIN GENE

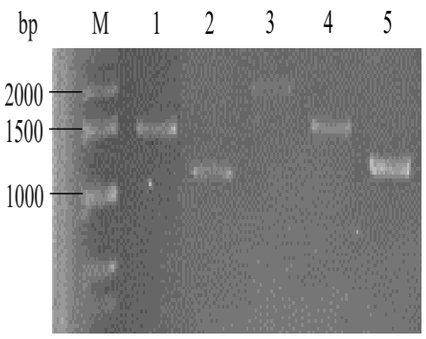
Slp is a monomolecular crystalline structure composed of protein or glycoprotein subunits with molecular masses ranging from 40 to 200 kDa (Sára & Sleytr 2000). In LAB, *Slp* plays a crucial role in the adhesion of intestinal tissues along with several other functional elements (Alp, Kuleaşan & Altıntaş 2020). Liu et al. (2021) proposed that *slp* is one of the bioactive components responsible for the anticancer effects in LAB.

Figure 4 shows the PCR results of the *slp* gene amplification, using the designated primers. The results indicate that different combinations of primers and annealing temperatures yielded five different bands of *slp* gene candidates, ranging in size from 1,300-2,000 bp. Furthermore, the PCR product was cloned and sequenced. The BLAST analysis showed a successful match to the targeted species *(L. plantarum)* and confirmed that the amplicon indeed originated from the *plantarum* species. Nevertheless, the BLAST search for this gene failed to generate a specific match for the *slp* gene in the NCBI database. This could be due to the absence of a known *slp* gene specific to *L. plantarum* in the NCBI database. Therefore, to gain a deeper understanding of the *slp* gene in *L. plantarum* IIA-1A5, it is necessary to compare the similarity of its sequence with other available *slp* gene sequences.

The gene sequence of *L. plantarum* IIA-1A5 was compared with other known *Lactobacillus slp* genes using multiple sequence analysis (MSA). The results are presented in Table 5 and showed an average percentage similarity ranging from 37% to 40% between *L. plantarum* IIA-1A5 and other *slp* genes. This indicates a moderate level of sequence similarity between the *slp* gene of *L. plantarum* IIA-1A5 and those of other *Lactobacillus* strains. Interestingly, when comparing the *slp* genes of different *Lactobacillus* strains within the same species, variations in sequence similarity were observed. Table 6 demonstrates the percentage comparisons from MSA. The values highlight the diverse sequences of the *slp* gene among different strains of the same *Lactobacillus* species. These findings suggest that the *slp* gene can vary even within a single species, emphasizing the genetic diversity among *Lactobacillus* strains.

Based on these results, it can be concluded that *L. plantarum* IIA-1A5 is likely to have its own *slp* gene, albeit with a relatively moderate level of sequence similarity to other known *Lactobacillus slp* genes. Previous studies have highlighted the key role of *slp* as a component for *lactobacilli* to exert their probiotic effects in the host, including pathogen inhibition as demonstrated by the *slp* of *L. acidophilus, L. helveticus,* and *L. plantarum* (Meng et al. 2014). Liu et al. (2021) indicated that *slp* also exhibits anticancer activity. Moreover, Li et al. (2011) reported that *slp* from *L. acidophillus* induced apoptosis in Caco-2 cells. Zhang et al. (2020) stated that *L. acidophilus* CICC 6074 *slp* has two mechanisms inhibiting HT-29 colon cancer cell proliferation and inducing apoptosis. The first pathway involves inhibiting the cell cycle at the G1 phase, while the second pathway involves inducing apoptosis in cancer cells by activating the FasL (Fas Ligand).

To date, most *slp* research has focused on specific strains such as *L. acidophilus*, *L. helveticus*, *L. crispatus*, and *L. brevis* (Meng, Zhang & Lu 2018). Therefore, there is still much to investigate about the various types and functions of *slp* in different *Lactobacillus* species, particularly in *L. plantarum* IIA-1A5.



M: DNA ladder; 1: Primers F4+R3, annealing temperature 36 °C; 2: Primers F4+R3, annealing temperature 36 °C; 3: Primers F4+R1, annealing temperature 41 °C; 4: Primers F4+R1, annealing temperature 41 °C; 5: Primers F4+R1, annealing temperature 41 °C

FIGURE 4. Electrophoresis result of the 2nd PCR

TABLE 5. MSA percentage comparisons of *L. plantarum* IIA-1A5 *slp* gene and known *Lactobacillus slp* gene in NCBI database

No	Name	*L. plantarum* IIA-1A5 *slp* gene sequence similarity (%)					
		Seq 1	Seq 2	Seq 3	Seq 4	Seq 5	Average
1	*Lactobacillus acidophilus* ATCC 4356 = NBRC 13951 SlpA [>X89375.1:287-1621]	39.18	37.26	39.17	38.85	37.68	38.49
2	*Lactobacillus acidophilus* ATCC 4356 = NBRC 13951 SlpB [>X89376.1:286-1656]	41.11	36.55	38.61	38.45	34.73	37.89
3	*Lactobacillus crispatus* JCM 5810 SlpA [>AF001313.1:142-1464]	42.37	35.88	38.46	38.29	36.29	38.26
4	*Lactobacillus helveticus* JCM1003 [>AB061776.1:74-1423]	39.10	40.69	40.10	40.10	36.77	39.35
5	*Lactobacillus kimbladii* Hma2N [>KC789972.1]	39.30	35.70	39.80	39.72	34.91	37.89
6	*Lactobacillus helsingborgensis* Bma5N [>KC776074.1]	36.43	34.80	41.03	41.15	35.29	37.74
7	*Lactobacillus acidophilus* LAB20 [>JN980173.1]	40.25	39.21	39.59	39.43	38.29	39.35
8	*Lactobacillus acidophilus* HAP50 [>AF250229.1]	39.06	34.59	37.38	37.30	37.52	37.17
9	*Lactobacillus crispatus* LMG 12003 SlpA [>AF253043.1]	41.43	39.96	40.80	40.89	34.71	39.56
10	*Lactobacillus crispatus* LMG 12003 SlpB [>AF253044.1]	40.66	35.53	37.33	37.16	35.46	37.23

TABLE 6. MSA percentage comparisons between known *Lactobacillus slp* gene in NCBI database

No	Name	*Slp* Gene Sequence Similarity (%)									
		1	2	3	4	5	6	7	8	9	10
1	*Lactobacillus acidophilus* HAP50 [>AF250229.1]	100	44.81	46.10	45.21	46.11	43.70	44.16	47.42	45.36	46.00
2	*Lactobacillus kimbladii* Hma2N [>KC789972.1]	44.81	100	72.43	48.07	46.89	47.13	48.14	49.10	50.31	48.00
3	*Lactobacillus helsingborgensis* Bma5N [>KC776074.1]	46.10	72.43	100	47.89	46.46	47.20	49.54	49.52	50.31	49.08

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4	*Lactobacillus crispatus* JCM 5810 SlpA [>AF001313.1:142-1464]	45.21	48.07	47.89	100	80.90	62.47	58.35	61.98	62.22	59.24
5	*Lactobacillus crispatus* LMG 12003 SlpB [>AF253044.1]	46.11	46.89	46.46	80.90	100	61.85	58.04	59.75	59.71	57.34
6	*Lactobacillus crispatus* LMG 12003 SlpA [>AF253043.1]	43.70	47.13	47.20	62.47	61.85	100	56.22	61.87	60.42	58.48
7	*Lactobacillus acidophilus* ATCC 4356 = NBRC 13951 SlpB [>X89376.1:286-1656]	44.16	48.14	49.54	58.35	58.04	56.22	100	68.37	57.40	63.40
8	*Lactobacillus acidophilus* ATCC 4356 = NBRC 13951 SlpA [>X89375.1:287-1621]	47.42	49.10	49.52	61.98	59.75	61.87	68.37	100	63.06	66.29
9	*Lactobacillus helveticus* JCM1003 [>AB061776.1:74-1423]	45.36	50.31	50.31	62.22	59.71	60.42	57.40	63.06	100	73.66
10	*Lactobacillus acidophilus* LAB20 [>JN980173.1]	46.00	48.00	49.08	59.24	57.34	58.48	63.64	66.29	73.66	100

Conclusions

Both intracellular and extracellular protein extracts from *L. plantarum* IIA-1A5 demonstrated anticancer properties. Specifically, the extracts showed cytotoxic and antiproliferative actions against MCF-7 breast cancer cells. Notably, the intracellular fraction demonstrated stronger anticancer activity than the extracellular fraction. Furthermore, the analysis of the *slp* gene shared a moderate sequence similarity with other known *Lactobacillus slp* genes. This illustrates the genetic diversity within the *L. plantarum* species and highlights the importance of further research to comprehend the specific role of the *slp* as an anticancer substance. To conclude, this study adds to the growing body of evidence demonstrating the potential of LAB, particularly *Lactobacillus plantarum* IIA-1A5, as a natural method for inhibiting cancer cell growth.

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