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Research Article

# Inhibition of Human Cervical Cancer Hela Cell Line by Meat-Derived Lactic Acid Bacteria of *Lactobacillus plantarum* IIA-1A5 and *Lactobacillus acidophilus* IIA-2B4

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## Abstract

**Background and Objective:** Two Indonesian lactic acid bacteria of *L. plantarum* I IA-1A5 and *L. acidophilus* IIA-2B4 were previously isolated from beef with some functional probiotic properties. Nevertheless, the possibility of these strains to have anticancer activity remains unknown. Current study aimed to evaluate the inhibitory properties of intra-and extracellular protein extracts of these two strains against cervical cancer HeLa cells. **Materials and Methods:** The intracellular and extracellular proteins extract from *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were collected and designated as IP-LP, IP-LA, EP-LP and EP-LA, respectively. The effect of these extracts on the viability and morphology of HeLa cells were observed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and confocal microscopy, respectively. **Results:** Both IP-LP and IP-LA inhibited HeLa cells in a concentration-dependent manner, with $IC_{50}$ values of 352.62 and 120.97 μg $mL^{-1}$, respectively. Meanwhile, the inhibition activity was also observed for EP-LP and EP-LA, *albeit* very low. The inhibition effect was also confirmed by morphological analysis under confocal electron microscopy which showed the changes in the cell shapes and numbers. **Conclusion:** Altogether, for the first time this study proposed that the probiotic isolated from Indonesian beef are promising to inhibit cancer cell lines.

Key words: Lactic acid bacteria, anticancer, *Lactobacillus plantarum* IIA-1A5, *Lactobacillus acidophillus* IIA-2B4, HeLa cells

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Cancer or a malignant tumor is one of the deadliest diseases in the world. In 2040, 28.4 million cancer cases are expected to be reported. This number is 47% higher than the cases in 2020 in which the transitioning countries have higher increment cases (64-95%) than transitioned countries (32-56%)[1]. One of the cancers which cause deaths worldwide is cervix cancer (cervical)[2,3]. The latest global cancer survey showed that cervical cancer is the second-highest incidence and mortality rates among women with an age-standardized incidence and mortality rate of 13.1 and 6.9 per 100,000, respectively. Arbyn *et al.*[4] reported that cervical cancer was the fourth most common cancer in women, ranking after breast cancer, colorectal cancer and lung cancer. Earlier, at least 500.000 women suffering from cervical cancer worldwide each year, which mostly happened in developing countries leading cause of mortality of 250.000 women[2,3].

Cervical cancer is associated with viral infection[5]. The most important cause of cervical cancer is persistent cervical infection with human papillomavirus (HPV)[6]. Healing the disease is usually performed with surgery, radiotherapy and chemotherapy[7]. Treatment with radiation has side effects[8] and may even increase the risk of other cancers[9]. Chemotherapy uses drugs to eliminate cancer cells. Doxorubicin, is one of the drugs that majorly uses chemotherapeutic, doxorubicin did not have upon ability in targeting cancer cells, this ability leading to poor distribution and therapeutic effects as well as serious undesirable side effects[10] Chemotherapy can inhibit the growth of cancer cells but also can harm normal cells[8]. Recently, many studies have focused on anti-cancer and antioxidative properties as protective adjuncts against a host of diseases[11,12]. The discovery and identification of new antitumor drugs with a low side effect on the immune system has become an essential goal in many studies of immunopharmacology[13,14]. These weaknesses lead to a new breakthrough cancer treatment with high effectiveness and minimal side effects.

One of the efforts of cancer treatment that can be performed is using Lactic Acid Bacteria (LAB)-based probiotics as it may produce some anticancer compounds inhibiting the cancer cell lines. Probiotic defined as additional nutrient consisted of living microorganism that can be considered functional food. The probiotic benefits are gained when it was consumed by human or animal in sufficient amount and viable in the intestine tract. It confers the health of a host animal by maintaining their intestinal microbial balance[15]. Some probiotics have been reported to possess certain anti-cancer and antitumor properties[16,17]. Nami[18] showed that the probiotic *L. acidophilus* has well-known anti-cancer activity besides its remarkable antimicrobial activity. Further, Nandhini and Palaniswamy[19] reported that *L. plantarum* JQ255472 may be considered as a more promising food component in terms of preventing cancer. Data from epidemiological and experimental studies have also indicated that the ingestion of certain LAB strains or fermented dairy products, might alleviate the risk of a certain type of cancers and inhibit the growth of tumours[17]. The habit of consuming probiotic in which their LAB yoghurt can prevent poisoning caused by free radicals that can cause cancer[20]. The aim of this study was to evaluate the effectivity of extract *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 against the proliferation of HeLa cells (Epithelioid Carcinoma Cervix). It is interesting that most anti-cancer compounds from LAB were reported to be secondary metabolites. To our knowledge, study on the involvement of protein-or peptide-based compounds on the anticancer properties remains limited.

Previously, two Indonesian probiotics, *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4, were isolated from the beef of Indonesian cattle, PeranakanOngole[21]. These bacteria had met requirements to be are categorized as strains of probiotics based on *in vitro* and *in vivo* studies[22]. Antibacterial activity and application of these probiotics were extensively studied. Some studies have revealed the advantage of *L. plantarum* 1A5, such as antibacterial activities against *E. coli, Staphylococcus aureus* and *Salmonella typhimurium*[23], retard the growth of pathogenic bacteria and has a high tolerance to low pH value[24-26]. *Lactobacillus acidophilus* 2B4 has also shown the ability to function as probiotic preventing diarrhea on EPEC infected rats and effect immunomodulatory by increasing the number of lymphocytes cells[23,27] yet their involvement in the inhibition of cancer cells proliferation has not been studied yet.

This report described the inhibition activity of intra-and extra-cellular protein compounds of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 against HeLa cervical cancer cells. The result leads to further promising applications of these probiotics for producing anti-cancer agents.

## MATERIALS AND METHODS

**Data source location:** The experiments were conducted at the laboratory of Animal Product and Technology, Faculty of Animal Science and the Primate Research Center of IPB University, Indonesia. The study was conducted during the period of September, 2018 to August 2019.

**Preparation of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4:** The LAB-based probiotic used in this research were *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4. These bacteria were firstly refreshed from the stock culture according to Arief[21]. Briefly, the stock culture was diluted 10-fold in De Man Rogosa Sharp Broth (MRSB) medium followed by incubation at 37°C for 24 hrs. This refresher process was performed 3 times. Final cultures obtained from this refreshment was used for the next stage.

**Extraction of intracellular protein extracts:** The extraction of intracellular fractions of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were performed according to Abed[28]. Briefly, the bacteria were firstly grown in 5 mL MRSB media overnight, followed by centrifugation at 14,000 rpm for 3 min at a temperature of 25°C and resuspended in 50 mM EDTA. A total of 33 mL lysozyme solution (40 mg $mL^{-1}$) was added to the suspension and incubated for 1 hr at 37°C. The suspension was centrifuged again as before and the supernatant was then harvested and used in the next step. The intracellular protein extracts from *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were then designated as IP-LP and IP-LA, respectively.

**Extraction of extracellular protein-based fractions:** The extraction of extracellular proteins of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were performed according to Abed[28]. The bacteria were firstly grown as described previously. The cells and medium were separated by centrifugation at 14,000 rpm for 3 min at a temperature of 25°C. The medium (supernatant) was then collected and dialysis in 50 mM EDTA for 24 hrs. Before use, the dialysis solution was centrifuged at 15,000 rpm for 20 min to remove the aggregated molecules. The extracellular protein extracts from *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were then designated as EP-LP and EP-LA, respectively.

**Analysis of protein content:** The protein content from the extracts were performed based on Lowry *et al.*[29]. Briefly, 1 mL suspension of sample was placed into the cuvette and add 1 mL solution of $Na_2CO_3$ 2% (w/v) in NaOH 0.1 N, NaK+tartrate 2% (w/v) and $CuSO_4.5H_2O$ 1% (w/v) with a ratio of 10: 0.5: 0.5. 3 mL of Folin-Ciocalteu added to the cuvette. After incubation for 10 min, the absorbance was monitored at the wavelength of 600 nm. For the standard curve, Bovine Serum Albumin (BSA) was used at a concentration of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg $mL^{-1}$.

**SDS page electrophoresis:** Electrophoresis was performed to observe the protein profile of the extracts. The electrophoresis was performed based on Laemmli[30] using 15% polyacrylamide gel, followed by silver staining (Sigma, St. Louis, MO, USA).

**Treatment of the HeLa cells by the extracts:** The treatment was loosely based on Chuah *et al.*[19]. The extracts (IP-LP, IP-LA, EP-LP and EP-NA) were prepared as before and adjusted into different concentrations before being added into HeLa cells. Each extract solution then diluted by adding Dulbecco's Modified Eagle Medium (DMEM) and buffer to obtain a final concentration in the microplate ranging from 3-200 µg. Into each microplate wells containing the HeLa cell of the previous stage (HeLa cell culture), was added 100 mL of the extract solution over the test as a treatment and added 100 mL of DMEM as control. The mixture in a microplate was incubated for 48 hrs in a $CO_2$ incubator at 37°C.

**Cytotoxicity test by MTT assay:** The test was performed based on Mosmann[31]. Briefly, following the HeLa cells incubation for 48 hrs in the absence or in the presence of extract solution, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was added into the culture yielding the changes of medium to yellow. The incubation was then prolonged for 4 hrs in a $CO_2$ incubator. Formazan crystals formed during the incubation was then harvested and diluted with 100 mL of 96% ethanol yielding the changes of solution color to be purple. The presence of formazan was then quantified under a microplate reader at a wavelength of 595 nm.

**Statistical analysis:** The data are presented as mean ± standard deviation from 3 independent replications. The one-way analysis of variance (ANOVA) was used to determine the differences among means with Tukey's method for a post hoc test and confidence interval of 95%.

## RESULTS

**Protein analysis:** Analysis of protein content using the Lowry method was conducted to determine the concentration of protein contained in the extracts (IP-LP, IP-LA, EP-LP and EP-NA). Figure 1(a-b) showed that the protein contents of IP-LP were statistically higher than that of EP-LA. Likewise, IP-LA also displayed higher protein content as compared to EP-LA. Further, the SDS Page Fig. 2(a-b) clearly showed that the protein bands of IP-LP and IP-LA were more visible than that

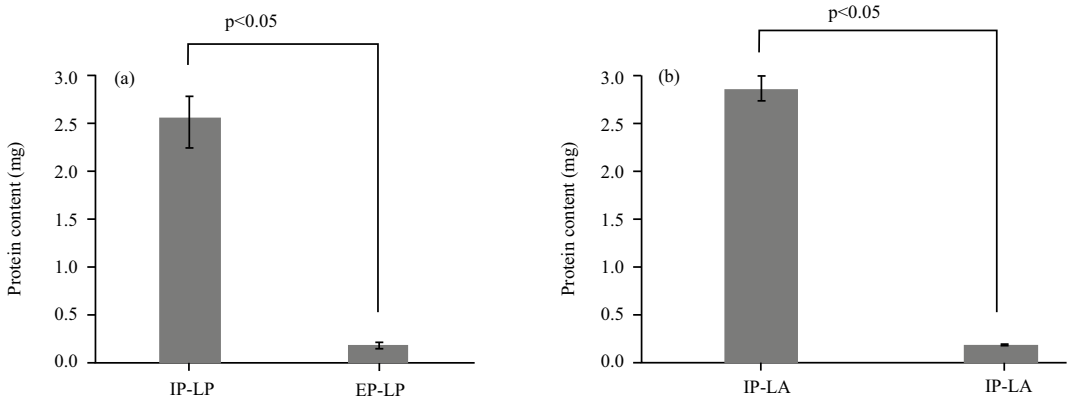


Fig. 1(a-b): Protein content of the extracts of (a) *L. plantarum* IIA-1A5 and (b) *L. acidophilus* IIA-2B4. IP-LP and EP-LP refer to intracellular and extracellular extracts of *L. plantarum* IIA-1A5, respectively. IP-LA and EP-LA refer to intracellular and extracellular extracts of *L. acidophillus* IIA-2B4, respectively

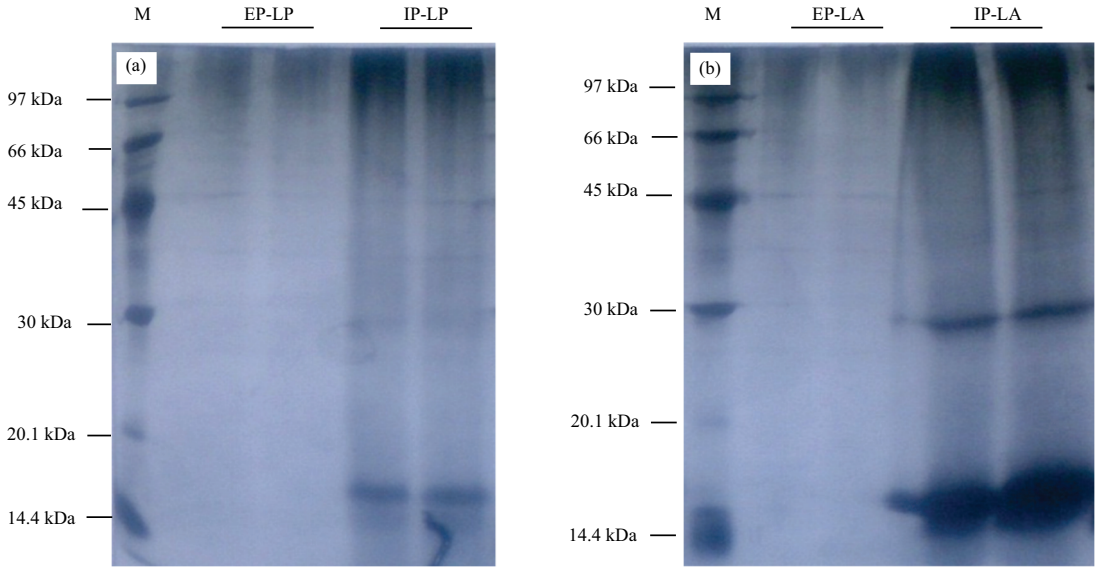


Fig. 2(a-b): 15% SDS Page of the extracts of (a) *L. plantarum* IIA-1A5 and (b) *L. acidophilus* IIA-2B4. IP-LP and EP-LP refer to intracellular and extracellular extracts of *L. plantarum* IIA-1A5, respectively. IP-LA and EP-LA refer to intracellular and extracellular extracts of *L. acidophillus* IIA-2B4, respectively. Each of extracts were run in two wells of the gel

of the protein bands of the extracellular extracts (EP-LP and EP-LA). In addition, the apparent size of the bands in the intracellular extracts (IP-LP and IP-LA) was wider than that of the extracellular extracts (EP-LP and EP-LA). The extracellular extracts from both strains (EP-LP and EP-LA) were shown to be dominated by the bands with the apparent sizes of >45 kDa.

**Inhibition of HeLa cells:** To examine the ability of the extract in retarding HeLa cells, an MTT assay was performed. The assay principally based on the ability of mitochondrial dehydrogenase reductase enzyme from active cell to transform the tetrazolium salt solution into a formazan product, Fig. 3(a-b) revealed that intracellular extracts of *L. plantarum* IIA-1A5 or *L. acidophilus* IIA-2B4 (IP-LP or IP-LA) remarkably inhibited the viability of HeLa cells in a concentration-dependent manner. The calculated $IC_{50}$ values for IP-LP and IP-LA were 352.63 and 120.98 μg $mL^{-1}$, respectively. These values were found to be statistically different ($p<0.05$), which indicated that the intracellular extract of IP-LA exhibited stronger inhibition than that of IP-LP.

Interestingly, Fig. 3(a-b) also showed that the inhibition of HeLa cells EP-LP were found to be relatively constant in the range of 3-200 μg $mL^{-1}$. Accordingly, the $IC_{50}$ value for EP-LP

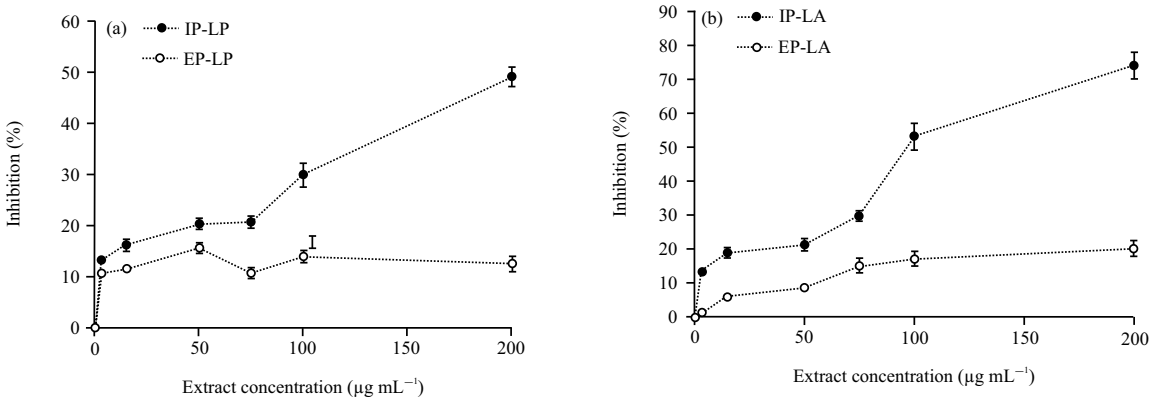


Fig. 3(a-b): Inhibition of HeLa cells by various concentrations of the extracts of (a) *L. plantarum* IIA-1A5 and (b) *L. acidophilus* IIA-2B4. IP-LP and EP-LP refer to intracellular and extracellular extracts of *L. plantarum* IIA-1A5, respectively. IP-LA and EP-LA refer to intracellular and extracellular extracts of *L. acidophillus* IIA-2B4, respectively

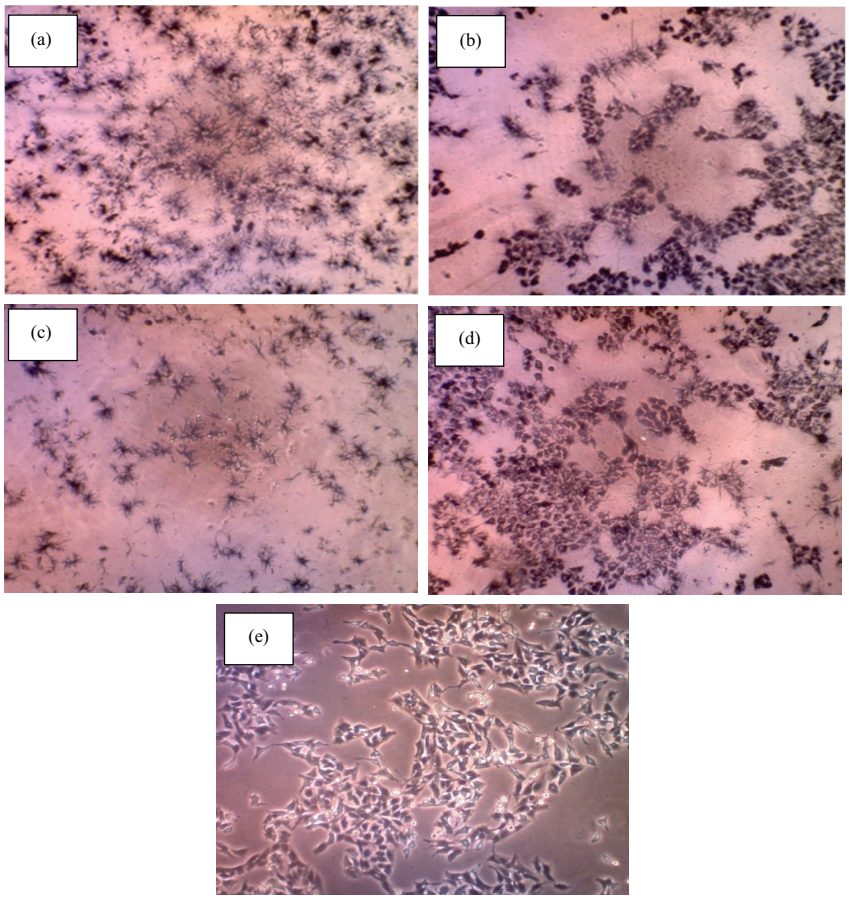


Fig. 4(a-e): Protein content of the extracts of (a) *L. plantarum* IIA-1A5 and (b) *L. acidophilus* IIA-2B4. IP-LP and EP-LP refer to intracellular and extracellular extracts of *L. plantarum* IIA-1A5, respectively. IP-LA and EP-LA refer to intracellular and extracellular extracts of *L. acidophillus* IIA-2B4, respectively. The morphology of HeLa cells in the presence of (a) Intracellular extract of *L. plantarum* IIA-1A5 (IP-LP), (b) Extracellular extract of L. plantarum IIA-1A5 (EP-LP), (c) Intracellular extract of *L. acidophillus* IIA-2B4 (IP-LA), (d) Extracellular extract of *L. acidophillus* IIA-1A5 (EP-LP) and (e) In the absence of extracts (control). The concentration of extracts used was 200 µg mL$^{-1}$ and observed under 100x magnification

was not feasible to be calculated from this result as it was not in a dependent-concentration manner. Nevertheless, EP-LA was shown to inhibit the HeLa cells in concentration dependent manner Fig. 3(a-b). The calculated $IC_{50}$ value for EP-LA was found to be 421.81 μg $mL^{-1}$, which is significantly higher ($p<0.05$) than the value for its intracellular extract, which indicated that the intracellular extract of IP-LA exhibited stronger inhibition than that of EP-LA.

**Morphological analysis of HeLa cells:** The difference of morphology HeLa cell after the addition of each extract (IP-LP or IP-LA or EP-LP or EP-LA) were shown in Fig. 4(a-e). As shown in Fig. 4(a-e), HeLa cells had been treated after the addition of MTT salt. Morphological differences of HeLa cells after being treated extracts on different types of bacteria with negative control showed the influence of the extract on the HeLa cells. The shape and growth of HeLa cells in the control treatment (0 μg $mL^{-1}$) seemed attached to all parts of the surface of the site and still stuck between cells and epithelial-like shape (polygonal). HeLa cells that had been getting treatment and inhibition occurred changes in its cell morphology including cell becomes more rounded, have regardless of the growth place, smaller or shrunken with lower cell density than the negative control.

## DISCUSSION

It is interesting to find that the protein content of IP-LP or IP-LA was higher than their extracellular fractions (EP-LP or EP-LA). This was also evidenced by the SDS-Page result which obviously showed that more visible bands were observed in the intracellular extracts than the extracellular extracts. This result is plausible since the number of protein secreted out of the cells are generally much less in compared to the proteins localized inside the cell (intracellular proteins). Nogueira *et al.*[32] reported that the secreted proteins in bacteria were about 5-6% of the total proteins only. Secretory proteins may include some enzymes, toxin as well as antimicrobial peptides. Secreted enzymes can be used to scavenge nutrients. Secreted toxins are involved in defense against protozoa, virulence towards humans and antagonistic interactions with other bacteria[33]. Sanchez *et al.*[34] also reported that extracellular proteins secreted by probiotic bacteria were found to be important for the attachment into the host mucosal cells. Despite there is no report on the number of secretory proteins of LAB, we believed that type and number of intracellular proteins are more varied as compared to secretory proteins. To be secreted out, proteins must be equipped with signal sequence either at their N-or C-terminal. This sequence is not required for intracellular protein hence the chance to keep reside inside the cells are relatively higher.

Further, IP-LP and IP-LA were shown to be remarkably able to reduce the viability of HeLa cells. HeLa is the most widely used model cell line for studying human cellular and molecular biology. These cells were taken from a woman named Henrietta Lacks, who had cervical cancer and died shortly afterwards and the HeLa cell line was then widely studied for the cervical cancer study[35,36]. The tie of intercellular apart can be caused by several factors, including enzymatic factors such as trypsin enzymes, proteases, collagenase[37]. The ability of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 to inhibit the HeLa cells line was in good agreement with some previous studies highlighting the inhibitory activity of some LAB against HeLa cells. Nami *et al.*[38] reported that *Lactobacillus plantarum* isolated from vaginal secretions exhibited anticancer activities against HeLa cervical cancer line. Similarly, some human milk-isolated *Lactobacillus* strains (*Lactobacillus casei* SR1, *Lactobacillus casei* SR2 and *Lactobacillus paracasei* SR4) were also able to inhibit the HeLa cell line[39]. Meanwhile, *L. casei* and *L. paracase* from cow milk were also previously reported to be able to inhibit the HeLa cell line[39]. In addition, Sungur *et al.*[40] reported that human vagina-isolated *L. gasseri* strains, G10 and H15, inhibits the HeLa cells proliferation. Nevertheless, those studies are all using extracellular extracts of the LAB. To note, no report so far for the anticancer activity of the intracellular extracts from the LAB groups. Therefore, this study is among the first study on the examination of intracellular extracts of the LAB against HeLa cancer cell line.

The apparent $IC_{50}$ value of the IP-LP indicated that this strain has weaker inhibition activity than IP-LA. This is acceptable as each species expressed a different type and amount of cellular components which might further differently involved in the inhibition activity. It should be noted that the extract is a mixture of various compounds (unpurified) that might interfere with each other. The concentration of the extract refers to a total concentration of whole compounds inside the extract and does not reflect the real concentration of the compound that responsible for anticancer activity. This might explain why the $IC_{50}$ value might relatively higher for the extract. While US National Cancer Institute (NCI) recommended the crude extracts to have less than 20 μg $mL^{-1}$ of $IC_{50}$ value (*in vivo*) to be considered as an anticancer compound, this standard applies

for plant-based compounds[41]. There are no standards, unfortunately, for the $IC_{50}$ value of bacterial based-compounds. Besides, the concentration of extract in this study refers to the protein content, not the whole compounds inside the extract.

To note, the extracellular extracts of both *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 (EP-LP and EP-LA) also demonstrated apparent inhibition activity against HeLa cell lines, albeit low activity. The concentration-dependent inhibition activity was obviously observed in *L. acidophilus* IIA-2B4 (EP-LA) but not for *L. plantarum* IIA-1A5 (EP-LP). The low activity might be due to the low protein composition in the extracellular extract as compared to the intracellular extract.

It is interesting to note that the highest inhibition activity by the IP-LP and IP-LA were 49.01 and 74.16%, respectively, at 200 μg $mL^{-1}$. Meanwhile, EP-LP and EP-LA were only 12.49 and 18.13%, respectively. Earlier, Luang-in *et al.*[42] reported that *L. plantarum* KK518 requires more than 300 μg $mL^{-1}$ of its extract to inhibit 50% of HeLa cells. Furthermore, Chuah *et al.*[19] reported that several strains of *L. plantarum* inhibited HeLa cells with the $IC_{50}$ values ranging from 18-24% (v/v). Meanwhile, *L. plantarum* RG11 was reported to have no ability to inhibit HeLa cells. This indicated that the concentration of *L. plantarum*'s extracts to inhibit the HeLa cells might vary depending on the strains.

The current study supports the accumulative studies on the promising anticancer activity of LAB groups. Some previous research also suggests LAB such as *Lactobacillus* have anticancer effects[16,43], *Lactobacilli* and *Bifido bacteria* were the most prominent of bacteria that used as probiotic bacteria and had gained wide interest in cancer research[44]. Some components of bacteria which have anti-cancer effects include peptidoglycan and the membrane component of the cell wall of LAB such as *Lactobacillus, Lactococcus, Streptococcus* and *Bifidobacterium*[16,45]. Kim *et al.*[46] used the whole extract *L. casei* ATCC393 for soluble polysaccharide components contained in the bacteria *Lactobacillus* strains to show inhibitory activity in cancer cell lines and cell wall (peptidoglycan) also have anti-cancer activity. Similarly, Liu and Pan[47] also reported that cytoplasmic extracts of *L. paracasei* ssp. *paracasei* NTU 101, *L. acidophilus* BCRC 14079, *L. salivarius* ssp. *salivarius* BCRC 14759 and *Bifidobacterium breve* BCRC 11846 exhibited remarkable anticancer activities. The inhibition magnitude was shown to be varied for different BAL strains. Choi *et al.*[48] using a bacterial culture *L. acidophilus* 606 and *L. casei* ATCC 393 is viewed of the proliferation of cancer cells.

While the anticancer study of LAB was widely studied, the mechanism by which this bacterium inhibited the cancer cell line remains to be further studied. Most anti-cancer drugs currently in use exert their effects via the induction of apoptosis[49]. Nevertheless, each of the LAB members utilized their own unique pathways of apoptosis induction. The cell-free culture supernatants of *Lactobacillus casei* SR1, *Lactobacillus casei* SR2 and *Lactobacillus paracasei* SR4 have anticancer activities through several mechanisms including downregulating BCL-2 and upregulating apoptotic genes (caspase3, caspase8, caspase9, BAX and BAD). The human vagina-isolated *L. gasseri* strains, G10 and H15, inhibits the HeLa cells proliferation through up-regulation of Bax and Caspase3. In addition, the strain also w as found to reduce TNF-α and increase IL-10, which leads to their anti-inflammatory effect on cervical cancer[40]. Meanwhile, *L. casei* 01 was reported to kill the cancer cells by decreasing the cell proliferation rate[50]. Furthermore, Nouri *et al.*[51] reported that HeLa cells treated by supernatants of *Lactobacillus rhamnosus* and *Lactobacillus crispatus* reduce the expression of CASP3 gene as well as MMP2 and MMP9, which causes an inhibitory effect on metastasis. These results indicated the ability of LAB extract used in this study has an anti-cancer effect which might be due to the capability of inhibiting cell proliferation and apoptosis inhibition. Nevertheless, it is also possible for LAB to utilize anti-proliferation and apoptosis induction effects. Ren *et al.*[52] proposed that the anticancer compounds may have different mechanisms, including anti-proliferation effect, cell cycle inhibition, angiogenesis inhibition, cell destruction that leads to necrosis and apoptosis induction. While the mechanism by which *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 remains to be further studied, this study provides an insight into the promising application of these Indonesian probiotic to be further developed as anti-cancer compound producers.

## CONCLUSION

This study demonstrated intra-and extracellular extracts of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 are capable of inhibiting the HeLa cancer cell lines. Intracellular extracts of both strains (IP-LP and IP-LA) displayed the highest inhibitory effect toward the cell line which might be due to the presence of more proteins and also other compounds as compared to that of extracellular extracts (EP-LP and EP-LA). This study also indicated that anticancer activity of *L. acidophilus* IIA-2B4 apparently higher than *L. plantarum* IIA-1A5 for both intra-and extracellular extracts. Altogether,

these bacteria are not only capable of improving the nutrition value of the food when it used in the food system but also promising to be harnessed as anti-cancer agents.

## SIGNIFICANCE STATEMENTS

This study discovers the anticancer activity of both intra-and extracellular extracts of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 against HeLa cervical cancer cells that can be beneficial for further studies and industrial applications. This study will help the researcher to uncover the critical areas of intra-and extracellular compounds responsible for the anticancer activity that many researchers have not explored. Thus, a new theory on the mechanism by which both strains inhibited HeLa cells may be arrived at.

## ACKNOWLEDGMENT

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## REFERENCES

1. Heer, E., A. Harper, N. Escandor, H. Sung, V. McCormack and M.M. Fidler-Benaoudia, 2020. Global burden and trends in premenopausal and postmenopausal breast cancer: A population-based study. Lancet Global Health, 8: e1027-e1037.
2. Wilson, C.M., S. Tobin and R.C. Young, 2004. The exploding worldwide cancer burden: The impact of cancer on women. Int. J. Gynecological Cancer, 14: 1-11.
3. Aggarwal, P., 2014. Cervical cancer: Can it be prevented? World J. Clin. Oncol., Vol. 5. 10.5306/wjco.v5.i4.775.
4. Arbyn, M., E. Weiderpass, L. Bruni, S. de Sanjosé, M. Saraiya, J. Ferlay and F. Bray, 2020. Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. Lancet Global Health, 8: e191-e203.
5. Jess, P.R.T., D.D.W. Smith, M. Mazilu, K. Dholakia, A.C. Riches and C.S. Herrington, 2007. Early detection of cervical neoplasia by raman spectroscopy. Int. J. Cancer, 121: 2723-2728.
6. Phongsavan, K., I. Gustavsson, L. Marions, A. Phengsavanh, R. Wahlström and U. Gyllensten, 2012. Detection of human papillomavirus among women in laos: Feasibility of using filter paper card and prevalence of high-risk types. Int. J. Gynecological Cancer, 22: 1398-1406.
7. Hahn, D.B. and W.A. Payne, 2003. Focus on Health. 6th Edn., Mc. Graw Hill, New York.
8. National Cancer Institute, 2012. Cancer Treatment. http://www.cancer.gov/ cervix/page8.
9. American Cancer Society, 2012. Cancer Facts and Figures 2012. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2012.html.
10. Hofland, K.F., A.V. Thougaard, M. Sehested and P.B. Jensen, 2005. Dexrazoxane protects against myelosuppression from the DNA cleavage-enhancing drugs etoposide and daunorubicin but not doxorubicin. Clin. Cancer Res., 11: 3915-3924.
11. Yang, C.S., J.M. Landau, M.T. Huang and H.L. Newmark, 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu. Rev. Nutr., 21: 381-406.
12. Iwai, K., N. Kishimoto, Y. Kakino, K. Mochida and T. Fujita, 2004. *In vitro* antioxidative effects and tyrosinase inhibitory activities of seven hydroxycinnamoyl derivatives in green coffee beans. J. Agric. Food Chem., 52: 4893-4898.
13. Duraikannu, K., K. Shameem Rani, R. Anithajothi, G. Umagowsalya and C.M. Ramakritinan, 2014. *In-vivo* anticancer activity of red algae (*Gelidiela acerosa* and *Acanthophora spicifera*). Int. J. Pharmaceut. Sci. Res., 5: 3347-3352.
14. Havsteen, B., 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol., 32: 1141-1148.
15. Fuller, R., 1991. Probiotics in human medicine. Gut, 32: 439-442.
16. Kim, J.Y., H.J. Woo, Y.S. Kim and H.J. Lee, 2002. Screening for antiproliferative effects of cellular components from lactic acid bacteria against human cancer cell lines. Biotechnol. Lett., 24: 1431-1436.
17. Kato, I., K. Endo and T. Yokokur, 1994. Effect of oral administration of *Lactobacillus casei* on antitumor responses induced by tumor resection in mice. Int. J. Immunopharmacol., 16: 29-36.
18. Nami, Y., N. Abdullah, B. Haghshenas, D. Radiah, R. Rosli and A.Y. Khosroushahi, 2014. Probiotic potential and biotherapeutic effects of newly isolated vaginal *Lactobacillus acidophilus* 36YL strain on cancer cells. Anaerobe, 28: 29-36.
19. Chuah, L.O., H.L. Foo, T.C. Loh, N.B.M. Alitheen and S.K. Yeap *et al*., 2019. Postbiotic metabolites produced by *Lactobacillus plantarum* strains exert selective cytotoxicity effects on cancer cells. BMC Complementary Altern. Med., Vol. 19. 10.1186/s12906-019-2528-2.
20. Pala, V., S. Sieri, F. Berrino, P. Vineis and C. Sacerdote *et al*., 2011. Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. Int. J. Cancer, 129: 2712-2719.

21. Arief, I.I., B.S.L. Jenie, M. Astawan, K. Fujiyama and A.B. Witarto, 2015. Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian local beef. Asian J. Anim. Sci., 9: 25-36.
22. Arief, I.I., Z. Wulandari, E.L. Aditia and M. Baihaqi, 2014. Physicochemical and microbiological properties of fermented lamb sausages using probiotic *Lactobacillus plantarum* IIA-2C12 as starter culture. Proc. Environ. Sci., 20: 352-356.
23. Arief, I.I., B.S.L. Jenie, M. Astawan and A.B. Witarto, 2010. The effectiveness of probiotic *Lactobacillus plantarum* 2C12 and *Lactobacillus acidophilus* 2B4 as antidiarrhea on rats. Media Peternakan, 33: 137-143.
24. Arief, I.I., B.S.L. Jenie, T. Suryati, G. Ayuningtyas and A. Fuziawan, 2012. Antimicrobial activity of bacteriocin from indigenous *Lactobacillus plantarum* 2C12 and its application on beef meatball as biopreservative. J. Indonesian Trop. Anim. Agric., 37: 90-96.
25. Arief, I.I., J.T. Suryati, Z. Wulandari and E. Andreas, 2013. Isolation and characterization of plantaricin produced by *Lactobacillus plantarum* strains (IIA-1A5, IIA-1B1, IIA-2B2). Media Peternakan: J. Anim. Sci. Technol., 36: 91-100.
26. Kia, K.W., I.I. Arief, C. Sumantri and C. Budiman, 2016. Plantaricin IIA-1A5 from *Lactobacillus plantarum* IIA-1A5 retards pathogenic bacteria in beef meatball stored at room temperature. Am. J. Food Technol., 11: 37-43.
27. Astawan, M., T. Wresdiyati, I.I. Arief and E. Suhesti, 2011. Hematology profile of *Rattuus norvegicus* infected by enteropathogenic *Escherichia coli* and fed probiotics. Media Peternakan, 34: 7-13.
28. Abed, T.A., 2013. Evaluation of methods for the extraction and purification of DNA of cultured *Lactobacillus colony* isolated from dairy products. Int. J. Appl. Microbiol. Biotechnol. Res., 1: 20-25.
29. Lowry, O.H., N.J. Rosebrough, A.L. Farrar and R.J. Randall, 1951. Protein measurement with the folinphenol reagent. J. Biol. Chem., 193: 265-275.
30. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
31. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.
32. Nogueira, T., M. Touchon and E.P.C. Rocha, 2012. Rapid evolution of the sequences and gene repertoires of secreted proteins in bacteria. PLoS ONE, Vol. 7. 10.1371/journal.pone.0049403.
33. Garcia-Garcera, M. and E.P.C. Rocha, 2020. Community diversity and habitat structure shape the repertoire of extracellular proteins in bacteria. Nat. Commun., Vol. 11. 10.1038/s41467-020-14572-x.
34. Sanchez, B., M.C. Urdaci and A. Margolles, 2010. Extracellular proteins secreted by probiotic bacteria as mediators of effects that promote mucosa-bacteria interactions. Microbiology, 156: 3232-3242.
35. Masters, J.R., 2002. HeLa cells 50 years on: The good, the bad and the ugly. Nat. Rev. Cancer, 2: 315-319.
36. Mirabelli, P., L. Coppola and M. Salvatore, 2019. Cancer cell lines are useful model systems for medical research. Cancers, Vol. 11. 10.3390/cancers11081098.
37. Freshney, R.I., 2000. Culture of Animal Cells: A Manual of Basic Technique. 4th Edn., John Wiley and Sons Inc., New York.
38. Nami, Y., N. Abdullah, B. Haghshenas, D. Radiah, R. Rosli and A.Y. Khosroushahi, 2014. Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium *Lactobacillus plantarum* 5BL. Microbiol. Immunol., 58: 492-502.
39. Rajoka, M.S.R., H. Zhao, Y. Lu, Z. Lian and N. Li *et al*., 2018. Anticancer potential against cervix cancer (HeLa) cell line of probiotic *Lactobacillus casei* and *Lactobacillus paracasei* strains isolated from human breast milk. Food Funct., 9: 2705-2715.
40. Sungur, T., B. Aslim, C. Karaaslan and B. Aktas, 2017. Impact of exopolysaccharides (EPSs) of *Lactobacillus gasseri* strains isolated from human vagina on cervical tumor cells (HeLa). Anaerobe, 47: 137-144.
41. Alabsi, A.M., K.L. Lim, I.C. Paterson, R. Ali-Saeed and B.A. Muharram, 2016. Cell cycle arrest and apoptosis induction via modulation of mitochondrial integrity by Bcl-2 family members and caspase dependence in *Dracaena cinnabari*-treated H400 human oral squamous cell carcinoma. BioMed Res. Int., Vol. 2016.
42. Luang-In, V., W. Saengha, B. Buranrat, S. Nudmamud-Thanoi, A. Narbad, S. Pumriw and W. Samappito, 2020. Cytotoxicity of *Lactobacillus plantarum* KK518 isolated from Pak-Sian dong (Thai fermented *Gynandropsis pentaphylla* DC.) against HepG2, MCF-7 and HeLa cancer cells. Pharmacogn. J., 12: 1050-1057.
43. Lee, J.W., J.G. Shin, E.H. Kim, H.E. Kang and I.B. Yim *et al*., 2004. Immunomodulatory and antitumor effects *in vivo* by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. J. Vet. Sci., 5: 41-48.
44. Wang, S.M., L.W. Zhang, R.B. Fan, X. Han and H.X. Yi *et al*., 2014. Induction of HT-29 cells apoptosis by *Lactobacilli* isolated from fermented products. Res. Microbiol., 165: 202-214.
45. Fichera, G.A. and G. Giese, 1994. Non-immunologically-mediated cytotoxicity of *Lactobacillus casei* and its derivative peptidoglycan against tumor cell lines. Cancer Lett., 85: 93-103.

46. Kim, S.N., W.M. Lee, K.S. Park, J.B. Kim, D.J. Han and J. Bae, 2015. The effect of *Lactobacillus casei* extract on cervical cancer cell lines. Contemp. Oncol., 19: 306-312.
47. Liu, C.F. and T.M. Pan, 2010. *In vitro* effects of lactic acid bacteria on cancer cell viability and antioxidant activity. J. Food Drug Anal., 18: 77-86.
48. Choi, S.S., Y. Kim, K.S. Han, S. You, S. Oh and S.H. Kim, 2006. Effects of *Lactobacillus* strains on cancer cell proliferation and oxidative stress *in vitro*. Lett. Appl. Microbiol., 42: 452-458.
49. Basse, B., B.C. Baguley, E.S. Marshall, W.R. Joseph, B. van Brunt, G. Wake and D.J.N. Wall, 2004. Modelling cell death in human tumour cell lines exposed to the anticancer drug paclitaxel. J. Math. Biol., 49: 329-357.
50. Liu, C.T., F.J. Chu, C.C. Chou and R.C. Yu, 2011. Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. Mutat. Res./Genet. Toxicol. Environ. Mutagen., 721: 157-162.
51. Nouri, Z., F. Karami, N. Neyazi, M.H. Modarressi and R. Karimi *et al*., 2016. Dual anti-metastatic and anti-proliferative activity assessment of two probiotics on HeLa and HT-29 cell lines. Cell J., 18: 127-134.
52. Ren, W., Z. Qiao, H. Wang, L. Zhu and L. Zhang, 2003. Flavonoids: Promising anticancer agents. Med. Res. Rev., 23: 519-534.