Anticancer Potential of *Lactobacillus acidophilus* IIA-2B4 Extracts Against MCF-7 Breast Cancer Cell Line

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ABSTRACT
Lactobacillus acidophilus IIA-2B4 is an indigenous lactic acid bacteria (LAB) extracted from Ongole Cross beef. Previous studies shown that this bacteria has probiotic properties such as antibacterial activity and antiproliferative effect on HeLa cervix cancer cell line. This study aimed to investigate the anticancer properties of *Lactobacillus acidophilus* IIA-2B4 against MCF-7 breast cancer cell line. Intracellular and extracellular extracts were obtained and MTT assay was used to determine the antiproliferative activity, along with doxorubicin as control. The concentration of extract used in MTT assay were 0, 15, 50, 100, and 200 ppm. The result shown that intracellular extracts have a better overall antiproliferative activity compared to extracellular extracts. Percentage of inhibition also increase along with higher level of concentration. Highest percentage of inhibtion by intracellular extract was 97.49% ± 1.197, meanwhile the highest percentage of inhibtion by extracellular extract was 97.03% ± 0.434. The lowest concentration of intracellular extract with antiproliferative activity similiar to that of doxorubicin was found in 50 ppm with 93.84% ± 2.220. The results indicate that both extracts have anticancer potential against MCF-7 breast cancer cell line and may provide as a natural alternative to treat and prevent cancer.

Keywords: *Anticancer, MCF-7, breast cancer, Lactobacillus acidophilus IIA-2B4.*

1. INTRODUCTION

Conventional methods to treat cancer such as surgery, radioteraphy, or chemotherapy were prone to negative side effects and other complications [1]. These issues have led to increased interest in approaches using anticancer compounds from natural sources, such as probiotics, as an alternative treatment with lower risks. Probiotics were microorganism that could confer health benefits when consumed in adequate amounts [2]. Probiotic lactic acid bacteria (LAB) were reported to have anticarcinogenic activities [3].

Lactobacillus acidophilus IIA-2B4 is an indigenous lactic acid bacteria (LAB) extracted from Ongole Cross beef. Previous studies have shown some probiotic properties such as antibacterial activity [4] and antiproliferative effect on HeLa cervix cancer cell line [5]. However, the antiproliferative activity against breast cancer were still unknown. This study aimed to investigate the anticancer properties of *Lactobacillus acidophilus* IIA-2B4 against MCF-7 breast cancer cell line.

2. MATERIAL AND METHODS

2.1. Material

Probiotic LAB Lactobacillus acidophilus IIA-2B4 (109 cfu mL-1) was obtained from IPB University Department of Animal Production and Technology laboratory collection. Breast cancer cell line MCF-7 used for the cell cytotoxicity assay was provided by IPB Primate Research Center (PSSP LPPM-IPB).

2.2 Methods

2.2.1. Preparation of bacterial extracts

The LAB was subjected to centrifugation at 6.000 x g at 4 ˚C for 10 minutes to isolate both pellets and supernatants. Isolated pellets were suspended in PBS solution with 10 mM EDTA. Approximately 16 µl of lysozyme (40 mg/ml) were added to the suspension. The suspension was subjected to sonication and centrifuged at 10.000 x g at 4°C for 20 minute, and the supernatants were collected to obtain the intracellular extracts.

Isolated supernatants from the initial centrifugation were filtered using Sartorius Minisart 0.22 µm membrane filter. The cell-free supernatant were subjected to dialysis using Wako dialysis membrane size 20 for 24 hours at 8°C in 0.02 M kalium phospate buffer (pH 6.2) with 10% of 10 mM EDTA. The final product of dialysis are the extracellular extracts. Lowry protein quantitation method was used to determine the protein concentration for each samples.

2.2.2. Anticancer activities

Anticancer activities were determined using MTT assay on MCF-7 breast cancer cell line. Intra and extracellular extracts were diluted with Dulbecco's Modified Eagle Medium (DMEM) and buffer into different protein concentration, namely: 0, 15, 50, 100, and 200 µg/mL. Doxorubicin with the same concentration were also used for treatment as a positive control. Extracts were inoculated into 96 wells microplate containing cancer cell and incubated for 48 h. MTT solution were added and the absorbance was measured at 595 nm wavelength. IC_{50} value were calculated using Graphpad Prism 8 by comparing the normalized absorbance percentage with log of concentration used.

2.2.3. Statistic

The experiment was arranged in a completely randomized factorial design with 2 factor (protein concentration and type of extract) and 3 replications. Data were analyzed by two-way analysis of variance using SPSS 25 (IBM). Significant ($P<0.05$) effects of treatments were calculated using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

MTT assay was performed to analyze the antiproliferative activity of an anticancer compound. The result showed that both intracellular and extracellular extracts of *Lactobacillus acidophilus* IIA-2B4 were capable to inhibit the growth of MCF-7 breast cancer cell in a dose-dependent response (57.31-97.49%) as stated in Table 1. The potency of inhibitory activity increased along with the higher number of concentration used. There was a significant difference ($P<0.05$) in response between 0, 15, and 50 µg/mL. However, the response for all samples in concentration higher than 50 µg/mL were not significantly differ. In cancer study, lower concentrations were more desirable because higher dose of anticancer compound may lead to increased risk of complication.

Intracellular extract of *Lactobacillus acidophilus* IIA-2B4 was significantly better at inhibiting cancer growth (92.23 to 97.49%) compared to extracellular extract (57.32 to 97.03%). Previous study on HeLa cancer cell also stated that intracellular extract of *Lactobacillus acidophilus* IIA-2B4 were the best in preventing cancer cell growth [5]. Bioactive compounds in intracellular extract may be responsible for these effect and its more various compared to the extracellular extract. However, the underlying bioactive compounds were still unknown and require further study. The inhibition of cancer cell growth by *Lactobacillus* has also been reported in other studies. Choi *et al*. [6] stated that *Lactobacillus acidophilus* extract decreases cancer cell survivability rate compared to controls for about 21%-28%. *Lactobacillus casei* isolated from dairy products had a significant anticancer effect on colorectal HCT116 cell lines by inhibiting proliferation and inducing apoptosis [7]. Functional food fermented with *lactobacillus* were also reported to had anticancer properties. Fermented milk containing five different strain of LAB (*B. infantis, B. bifidum, B. animalis, L. acidophilus,* and *L. paracasei*) inhibited the growth of MCF-7 breast cancer cell line [8]. Camel milk fermented by *Lactobacillus lactis* exhibited antiproliferative activities againts Caco-2, MCF-7, and HeLa cancer cell line [9]. In a case-control study in The Netherlands, consumption of fermented milk products was significantly less among 133 breast cancer patients than among 289 healthy controls, suggesting that LAB can prevent breast cancer [10].

Doxorubicin is a non-selective, conventional drug used to treat various kind of cancer. It had a very high antiproliferative capabilities even on a lower concentration. The disadvatange of using doxorubicin was the inability to diferentiate between normal cells and cancer cells due to lack of active targeting capability [11]. Thus, healthy cells may be at risk at prolonged usage. MTT assay result showed that

Table 1. Antiproliferative activites of bacterial extracts (%)

Sample	Concentration (µg/mL)				
	0^{A}	15^{B}	50^{C}	100^{C}	200^{C}
L.a. – Intra^{b}	$0^{A,b}$	$92.23\pm3.25^{B,b}$	$93.84\pm2.72^{C,b}$	$95.63\pm1.18^{C,b}$	$97.49\pm1.47^{C,b}$
L.a. – Extra^{a}	$0^{A,a}$	$57.32\pm12.13^{B,a}$	$92.19\pm1.33^{C,a}$	$93.29\pm1.28^{C,a}$	$97.03\pm0.53^{C,a}$
Doxorubicin^{b}	$0^{A,b}$	$93.75\pm1.12^{B,b}$	$94.27\pm0.84^{C,b}$	$95.44\pm1.53^{C,b}$	$93.80\pm0.49^{C,b}$

$^{A–C}$Means in the same column with different superscripts differ ($P < 0.05$).
$^{a–b}$Means in the same row with different superscripts differ ($P < 0.05$).

intracellular extract and doxorubicin weren't significantly different, suggesting that *Lactobacillus acidophilus* IIA-2B4 extract could be used as a safer alternative to treat cancer.

IC_{50} value represent the required amount of concentration to inhibit half of cancer cell growth. Atjanasuppat *et al.* [12] classified anticancer potency of a compound based on its IC_{50} values into four different categories, namely: strong (<20), moderate (>20-100), weak (>100-1000), and inactive (>1000). Absorbance result from MTT assay were normalized and compared with log of concentration to determine the IC_{50} value as stated in Table 2. Based on the classification before, all samples could be categorized with strong anticancer activity. Although both extracts were on the same categories, intracelullar extracts (5.788 µg/mL) performed better with a lower IC_{50} value than their extracellular counterparts. The lower IC_{50} value is more desirable because higher concentration of compound used may cause complication with healthy cells. The IC_{50} value of doxorubicin (5.401 µg/mL) was similiar with intracelullar extracts, thus suggesting that intracellular extract of *Lactobacillus acidophilus* IIA-2B4 has a promising potential as an alternative treatment for breast cancer.

Table 2. IC_{50} value of bacterial extracts (µg/mL)

Sample	IC_{50}
L.a. – Intra	5.788
L.a. – Extra	12.490
Doxorubicin	5.401

4. CONCLUSION

Both intracellular and extracellular extracts were capable to shown antiproliferative activities against MCF-7 cell. The responses were dose-dependent and better performance were displayed in higher concentration. Intracellular extract of *Lactobacillus acidophilus* IIA-2B4 was better at inhibiting cancer growth than their extracellular counterparts, and the IC_{50} value was similiar to that of doxorubicin.

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