American Journal of Biochemistry and Biotechnology

Original Research Paper

Bioactive Compounds from Sponge Associated Bacteria: Anticancer Activity and *NRPS-PKS* Gene Expression in Different Carbon Sources

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Article history
Received: 16-08-2017
Revised: 29-08-2017
Accepted: 25-09-2017

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Abstract: Sponge associated bacteria with diverse *NRPS-PKS* genes could be the alternative source of bioactive compounds. This study aimed to analyze the activity as well as the regulatory mechanism regarding the anticancer properties derived from these bacteria against cancer cell lines. Extraction of bacterial metabolites using ethyl acetate solvent resulted yield ranging from 0.003 to 0.016% (w/v). Six extracts of bacterial metabolites showed markedly cytotoxic activity against 8 human hematopoietic cancer cell lines, with IC_{50} value 27.24 to 533.73 μg mL^{-1} in a dose dependent response, as assessed by MTT test. Supporting those results, reduction of cell density, morphological changes including cell shrinkage and formation of the apoptotic body of cancer cells lines, were found. As it might be due to the inhibitory effect of bacterial extract treatment. Apoptosis assay determined by annexin V-FITC apoptosis analysis showed that apoptotic cells were induced by bacterial extract after 6 h of treatment. Thus suggesting that the extract could be one of the external signals that activate the signal transduction pathway of apoptosis, especially in MOLT4 cells, acute lymphoblastic leukemic cell lines. Cell cycle analysis using propidium iodide revealed that the extract HAA 01 caused cell cycle arrest, since most of the MOLT4 cells were accumulated in Sub-G1 phase after 24 h of extract treatment. Interestingly, glycerol, lactose and amylum were the more preferable carbon source to produce anticancer compounds, than glucose. Our study indicates that anticancer activity of HAA 01 (identified as *Bacillus subtilis* based on API identification) extract is highly correlated with *NRPS-PKS* gene expression which is likely regulated by the type of carbon source.

Keywords: Anticancer Activity, Sponge Associated Bacteria, Nonribosomal Peptide Synthetase (NRPS), Polyketide Synthase (PKS), Carbon Source

Introduction

Cancer is one of the serious diseases causing mortality worldwide. The disease caused by genetic mutations that cause uncontrolled cell growth and can spread throughout the body (Bhatt *et al*., 2010). World Health Organization (WHO, 2017) reported that cancer is responsible for 8.8 million deaths in 2015. The number of cancer prevalence is expected to increase in the next decade. Therefore, the discovery of a new anticancer drug would be important in cancer treatment.

Principles of cancer treatment are to reduce the number of cancer cells by inducing apoptosis and inhibiting cell proliferation (Kuno *et al*., 2012). Chemotherapy plays an important role to treat cancer, but it still not effective due to many side effects, such as experienced are hyperuricemia, hair loss, nausea and vomiting, depression of bone marrow, anemia, bleeding, alopecia and mucositis (Hassan, 2012). The development of a new anticancer that lack of side effect is essential to effectively reduce the cancer prevalence. Screening of natural products is expected to

obtain a bioactive compound with high anticancer activity with minimum side effects.

Sponge associated bacteria could be the alternative source of bioactive compounds. In the previous study, exploration of secondary metabolites extracted from bacteria associated with sponges *Callyspongia* sp, *Petrosia* sp and *Stylotella* sp. (from Bira Island, Kepulauan Seribu, Jakarta), *Haliclona* sp. and *Jaspis* sp. (from Raja Ampat Island, Papua) has been done. They have some of medicinal properties, including antimicrobial activity (Tokasaya, 2012; Yoghiapiscessa *et al*., 2016), protease inhibitor (Wahyudi *et al*., 2010), antioxidant and anticancer on HeLa cell lines (Utami *et al*., 2014; Safari *et al.*, 2016) and leukemic cell lines (Karwati *et al*., 2015). Based on those potential characters, thus in this study, we attempted to understand the activity as well as the regulatory mechanism of the anticancer properties derived from these sponge associated bacteria towards human hematopoietic cancer cell lines. It is worth noting that the genes encoding bioactive compounds, Nonribosomal Peptide Synthetase (*NRPS*) and Polyketide Synthase (*PKS*) gene have been detected in some of the bacterial isolates (Safari *et al*., 2016). Therefore, this study is essential to confirm the involvement of these genes in the synthesis of bacterial anticancer compounds. In addition, synthesis of bioactive compounds can be affected by carbon source in the growth medium (Ruiz *et al.*, 2010). Carbon sources are often involved in transcriptional activation or repression of genes (Uguru *et al*., 2005), therefore modification of medium carbon source needs to be done to provide the suitable condition to produce anticancer compounds. Thus, the study concerning the effect of carbon source on the expression of the anticancer compound synthetic pathway was conducted.

Materials and Methods

Culture of Sponge Associated Bacteria

Each bacterial isolate was cultured in 1 L Sea Water Complete (SWC) medium (bacto peptone 5 g, yeast extract 1 g, glycerol 3 mL, artificial sea water 750 mL and distilled water 250 mL) and incubated in a shaker incubator at 100 rpm for 3 days at 28°C.

Extraction of Bacterial Metabolites

Bacterial extracts were prepared by using ethyl acetate. Extractions were conducted based on the previous methods (Utami *et al*., 2014; Safari *et al.,* 2016; Karwati *et al*., 2015) but with several modifications. Following addition of 1 L ethyl acetate to the bacterial culture, the culture and solvent solutions were then shaked continuously for 20 min in a separating funnel. The solvent layer was evaporated at 40°C. Then, the bacterial extracts were stored below 5°C until used. The bacterial extract was solubilized in Dimethyl Sulfoxide (DMSO) before used.

Anticancer Activity Assay

Cancer cell lines used in this study were K562 (chronic myelogenous leukemia), Nalm6 (lymphoma), MOLT4 (acute lymphoblastic leukemia), Raji (Burkitt's lymphoma), Daudi (Burkitt's lymphoma), CEM6 (human T leukemic cell lines), HL60 (acute promyelocytic leukemia), U937 (histiocytic lymphoma) from RIKEN Cell Bank, Japan. A cytotoxic property of six potential bacterial extracts was analyzed by Microculture Tetrazolium Test (MTT) against cancer cell lines (Thakur *et al*., 2005). 50 µL of cell suspension (5×10^3 cells/well) was plated into 96-well plates containing 50 µL Roswell Park Memorial Institute Medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS), 2% L-glutamine and 1% streptomycin penicillin in various extract concentrations (0, 25, 50, 100, 200, 400, 800 µg mL^{-1}) and incubated at 35°C in 5 % CO_2 condition for 48 h. The medium without extracts was used as negative control and doxorubicin was used as a positive control. After 48 h, 10 µL of Cell Counting Kit (CCK-8) were added to each well and incubated at 35°C for 4 h, then measured the Optical Density (OD) at 450 nm using microplate reader MTP-450 (Corona Electric, Japan). The Inhibition of cell growth was determined using the following formula:

$$\%\,Inhibition = \frac{OD\,of\,control\,cells - OD\,of\,treated\,cells}{OD\,of\,control\,cells}\times 100\%$$

This assay was performed in triplicate. The IC_{50} value was determined by the linear regression.

Apoptosis Assay

The effect of the extract on cell apoptosis was analyzed by using Annexin V-FITC Apoptosis Detection Kit (ApoScreen®, Southern Biotech) following the previous method with some modifications (Karwati *et al*., 2015). Approximately 1×10^6 MOLT4 cells were incubated with 100 µg mL^{-1} of bacterial extracts and 5 µg mL^{-1} of doxorubicin for 6 h. Apoptosis assay was carried out according to the manufacturer's protocol.

Cell Cycle Assay

Cell cycle analysis was conducted as described previously with some modifications (Karwati *et al*., 2015). MOLT4 cells were seeded at a cell density of 1×10^6 cells/well in 24-well plates and incubated with 100 µg mL^{-1} of bacterial extracts and 5 µg mL^{-1} doxorubicin for 24 h at 37°C in 5% CO_2 condition. After treatment, treated cells were centrifuged and the pellets were then washed three times with Phosphate Buffer Saline (PBS) prior to addition of 0.9 mL staining solutions. Staining solution for 10-11 samples were prepared by mixing 4.5 mL propidium iodide stock

solution (0.1 mg mL^{-1} DDW), 1 mL Na-citrate stock solution (0.012 g mL^{-1} DDW), then 0.45 mL^{-1} RNase (10 mg mL^{-1}), 0.1 mL 10% triton X-100, 3.95 mL DDW). Cells suspension were then mixed by using vortex and incubated for 10 min on ice prior to addition of 0.1 mL 1.5 M NaCl and filtered through a nyron mesh. Samples were analyzed by flow cytometry (Accuri™ C6, USA). This assay was carried out in triplicate. The output data analyzed by FlowJo v10 software.

Carbon Source Modification and NRPS-PKS Gene Expression Analysis

The most potential bacterial isolate was cultured in SWC medium containing 0.3% with different carbon sources as glycerol, glucose, lactose and amylum. The bacterial RNA was extracted by using Nucleospin® RNA Plus Kit following to the manufacturer's instructions. PKS-NRPS gene expression analysis was carried out by one step quantitative reverse transcription PCR (qRT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene (Primer GAPDHF: GTT CCC ACT GTC GAT GTC TCA, GAPDHR: CCC TTC ATC TTG CCC TCA GA) (Petriccione *et al.*, 2015) was used as the control. Primer for *PKS* (PKS012F: ATC CCA GAC CCC GTA TGT CA; PKS012R: GAG TGC GAT GTT GCG TTA GC) and *NRPS* (NRPS011F: CGC TGC AAC CTT CGA TTC AG; NRPS011R: GTC CCG ATT GAT CCG GGT TT) were designed using PRIMER 3. PCR mix was performed according to Takara Onestep SYBR manual in a total reaction volume of 10 µL, consist of 5 µL of 2X one step SYBR RT-PCR buffer 4, 0.6 µL TaKaRa Ex Taq HS Mix, 0.2 µL PrimeScript PLUS RTase mix, 0.4 µL PCR forward primer (10 µM), 0.4 PCR reverse primer (10 µM), 0.2 µL ROX reference dye, 1 µL total RNA (100 ng) and adjusted with RNase Free dH_2O to 10 µL. Cycling parameters for reverse transcription are 42°C for 5 min and 95°C for 10 s, followed by 40 cycles of PCR reaction with DNA denaturation at 95°C for 5 s, primer annealing 55°C for 30 s. The evaluation of expression levels of mRNA was determined by calculating their $2^{-\Delta\Delta CT}$ values.

Anticancer Analysis of Bacterial Metabolites from Different Type of Carbon Sources

The extracts were prepared from the bacterial isolates cultured in different carbon sources (0.3% of glycerol, glucose, lactose and amylum). The cytotoxic property of the bacterial extracts was then analyzed by Microculture Tetrazolium Test (MTT) against MOLT4 cells, as described previously.

Biochemical Identification

The biochemical characters of the most potential isolate were investigated by using API system (BioMerieux, Marcy l'Etoile, France). 50 CHB strips was used for bacterial identification. Prior to inoculation of API system, the bacterial isolate was cultured in Luria Bertani Agar for 24 h. Then, Biochemical identification was conducted by following API's Protocol. The binary data of biochemical characters was then entered into the Apiweb software (BioMerieux).

Results

Crude Extracts of Bacterial Secondary Metabolites

Secondary metabolites of 6 bacterial isolates were extracted using ethyl acetate and resulting crude extracts as yield, ranging from 0.003 to 0.016% weight per volume. The crude extracts have various in weight and color. The extract derived from STIL 33 isolate has resulted the highest yield percentage.

Anticancer Activity of Sponge Associated Bacteria

Cytotoxic properties of 6 bacterial crude extracts were determined by MTT test. The capability of 6 bacterial crude extracts to inhibit the proliferation of cancer cells was found diverse, as shown by the various IC_{50} value ranging from 27.24±11.57 µg mL^{-1} to 533.73±29.1 µg mL^{-1} (Fig. 1). Bacterial extracts were able to inhibit all cancer cell lines tested in a dose dependent response. However, the IC_{50} value of those extracts was higher than doxorubicin as a positive control (IC_{50} value: 13.26±0.41 µg mL^{-1} to 32.76±2.71 µg mL^{-1}). From the MTT assay, we found four potential extracts with highest IC_{50} value were detected, including HAA 01, HAL 74, HAL 13, STIL 37. Among them, the extract of HAA 01 showed the best anticancer activity on some of the cancer cell lines, with the highest IC_{50} value (27.24±11.57 µg mL^{-1}) against CEM6, human T leukemic cell lines. Anticancer activity of the bacterial metabolites extract could also be observed from the alteration of cell density and morphology of cancer cells. The morphological alteration was observed as cells underwent a change from polygonal to unanimous form, considered apoptotic cells. Apoptotic cells can be easily observed following the application of 200 µg mL^{-1} extract. The application of higher concentration caused significant reduction in cell density (Fig. 2).

Bacterial Extracts Induces Apoptosis of MOLT4 Cells

To confirm the results of MTT assay, four potential bacterial extracts with the lowest IC_{50} value were subjected to apoptosis assay of MOLT4 cells by flow cytometry. The effectiveness of the extract in inducing cells apoptosis could be determined by the depletion of that viable cells (Table 1). HAA 01 showed the strongest effect based on its ability to reduce the number of viable cells to nearly 70.5±0.8%. Whereas, about 27.8±1.3% and 1.7±0.6% cells were found in the early apoptotic phase and late apoptotic phase, respectively. The cell population is significantly different with untreated cells that have more viable cells (98.7%) and DMSO treatment (98.6%).

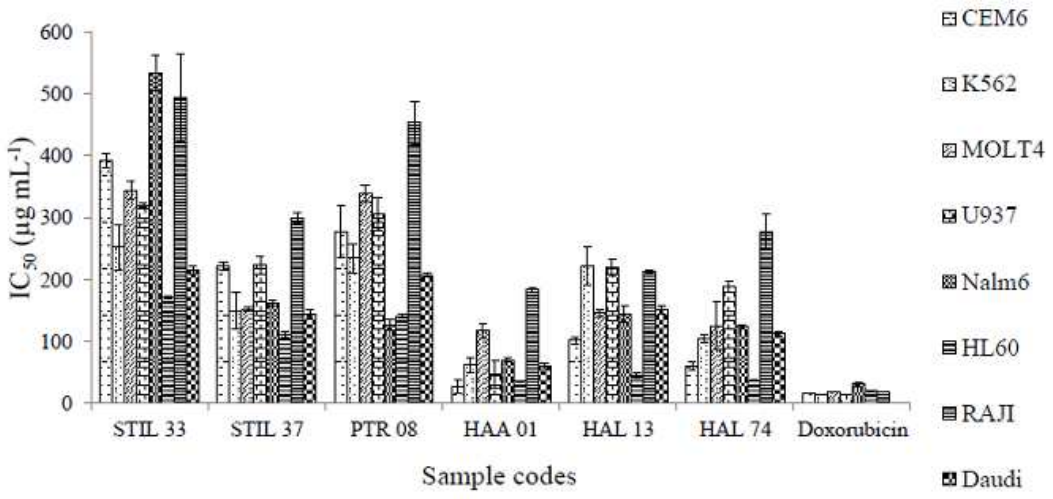

Fig. 1. Anticancer activity of 6 bacterial extracts on some of the human hematopoietic cancer cell lines. Anticancer activity was measured by using MTT assay in three independent replications. Doxorubicin was used as positive control. CEM6 (human T leukemic cell lines), K562 (chronic myelogenous leukemia), MOLT4 (acute lymphoblastic leukemia), U937 (histiocytic lymphoma), Nalm6 (lymphoma), HL60 (acute promyelocytic leukemia), Raji (Burkitt's lymphoma), Daudi (Burkitt's lymphoma)

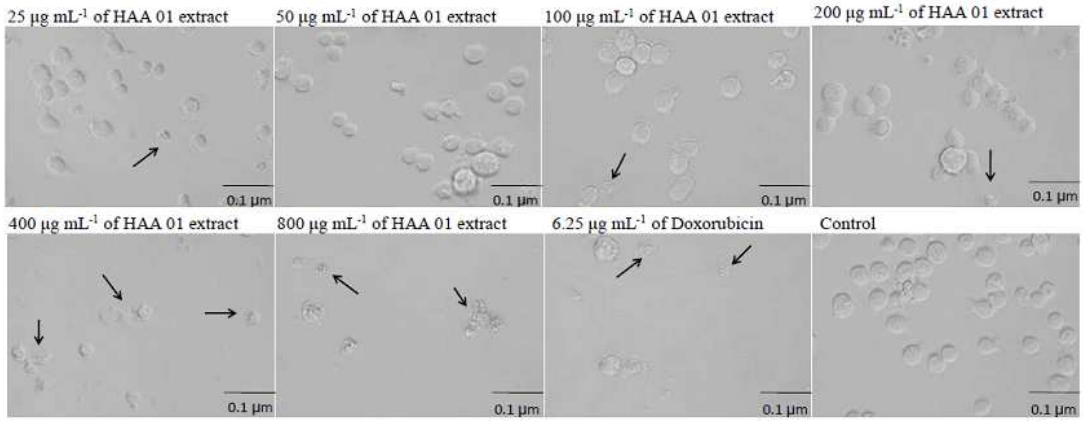

Fig. 2. Density and morphology of CEM6 cells following 48 hours of extract treatment. Cell morphology was observed in phase contrast microscope 400.0x. Cells were exposed to 25, 50, 100, 200, 400, 800 µg mL^{-1} of HAA 01 extracts. Doxorubicin (6.25 µg mL^{-1}) and without extract treatment were used as the positive and negative control, respectively. Arrow represents apoptotic cells

Table 1. Effect of bacterial extracts on the apoptosis of MOLT4 cells

Samples	Viable cells (%)[a]	Early apoptotic cells (%)[a]	Late apoptotic cells (%)[a]	Dead cells (%)[a]
Untreated	98.7±0.2	1.1±0.1	0.2±0	0±0
DMSO	98.6±0.6	1.2±0.7	0.2±0.2	0±0
Doxorubicin	73.9±2.6	25.1±3.6	1.1±1.2	0±
HAA 01	70.5±0.8	27.8±1.3	1.7±0.6	0±0
HAL 13	87.9±1.8	11.8±1.8	0.3±0.1	0±0
HAL 74	78.7±2.9	20.8±3	0.4±0.1	0.1±0
STIL 37	95.2±1.5	4.3±1.7	0.4±0.1	0.1±0

MOLT4 cells were incubated with 100 µg mL^{-1} bacterial extracts and 5 µg mL^{-1} doxorubicin for 6 h in triplicates, [a]mean±SD

The Inhibitory Effect of Bacterial Extract on Cell Cycle Progression

Cell cycle progression was determined based on DNA content which proportional with PI fluorescence. The apoptotic cell was represented as sub-G1 population. The cells treated with samples had different DNA content compared with untreated cells. As shown in Fig. 3, there was the significant decrease in the number of DNA content in G1, S and G2-M and

accumulation of DNA content in sub-G1 after extract (100 µg mL^{-1}) and doxorubicin (5 µg mL^{-1}) treatments for 24 h. HAA 01 treatment caused significant alteration particularly by arresting the MOLT4 cells cycle at G1 phase, since 38.5% cells were found in sub-G1 phase, 15.2% cells at G1, 4.49% at S, 1.34% at G2-M and more than 40% cells were non-viable after treatment. Meanwhile, in untreated cells, most of the cells were found in G1 phase (58.9%), S phase (24.1%), G1-M phase (13.6%) and only a few cells in Sub-G1 (2.32%).

NRPS-PKS Gene Expression in Different Carbon Sources

We studied the effect of different carbon sources in the mRNA levels of *PKS* and *NRPS* genes of the most potential isolate, HAA 01. In this analysis, glycerol was employed as a control since this particular carbon source was used in former assays. As shown in Fig. 4, expression of *PKS* gene was down-regulated in glucose, lactose and amylum as compared to that glycerol. Interestingly, lactose significantly up-regulated the expression of *NRPS* genes with 1.2 fold increase compared to glycerol, on the other hand, glucose and amylum repressed the expression of *NRPS* genes.

Anticancer Activity of Bacterial Extract from Different Carbon Sources

The extract from HAA 01 grown in different carbon sources was further tested for its anticancer activity against MOLT4 cells. The IC_{50} value among the extracts was various. Bacterial extracts from lactose and glycerol exhibited the best IC_{50} value, at 112.03±2.15 µg mL^{-1} and 149.63±43.47 µg mL^{-1}, respectively. However, no differences were found between the IC_{50} value of extract from glycerol and lactose. In contrast, glucose has the lowest IC_{50} value than other carbon sources at 525.95±5.74 µg mL^{-1}, which was 3 and 2 times lower than lactose and amylum (Fig. 5).

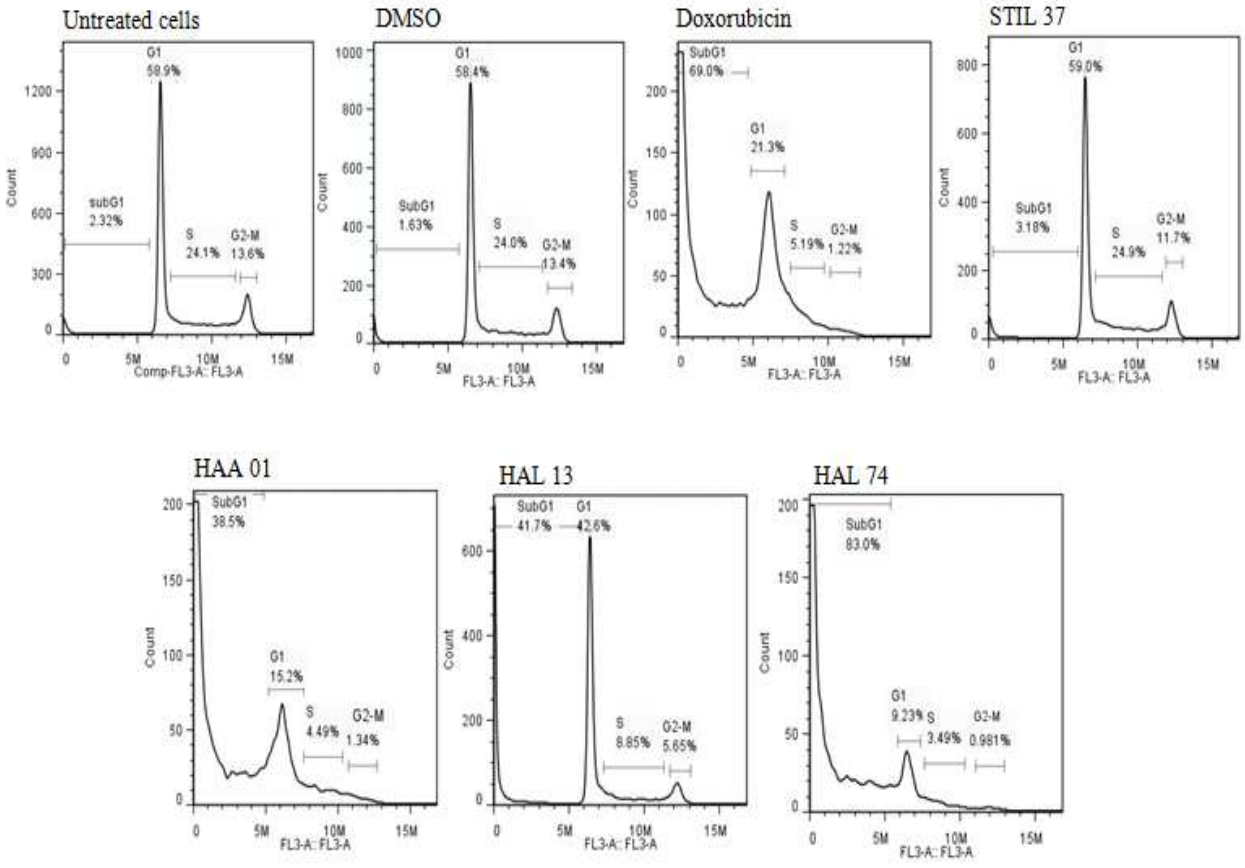

Fig. 3. The effect of bacterial extracts (100 µg mL^{-1}) on the cell cycle distribution in MOLT4 cells after 24 h treatment. Analysis of cell number was presented as the percentage of each cell cycle phase relative to total phases. Doxorubicin (5 µg mL^{-1}) was used as positive control while untreated cells and DMSO treatment were employed as the negative control

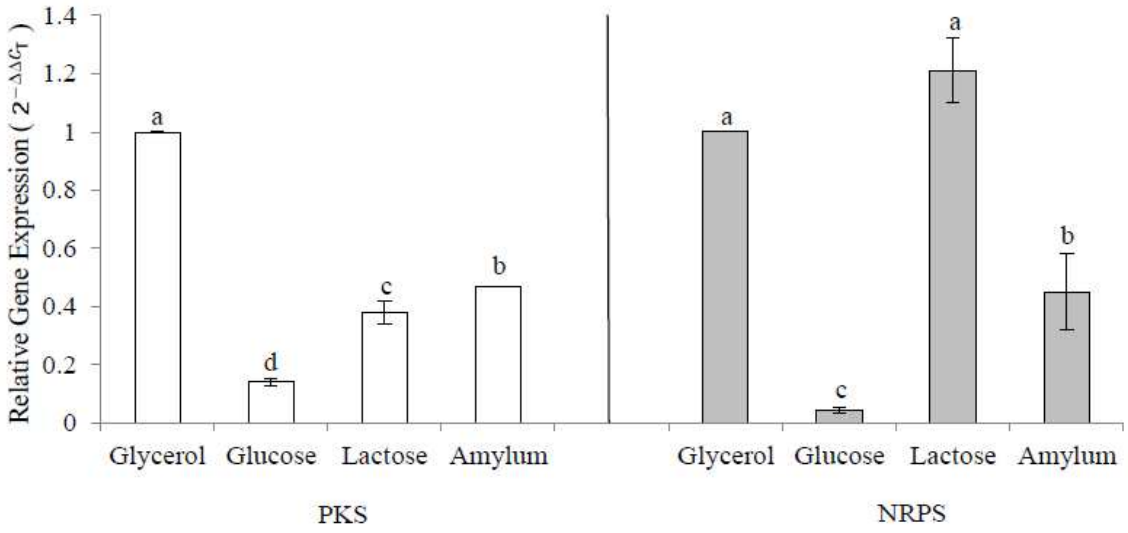

Fig. 4. *NRPS-PKS* gene expression of HAA 01 strain incubated in the liquid medium containing different carbon sources. The *NRPS* and *PKS* mRNA levels were determined by qRT-PCR experiment. Error bars represent standard deviations. Different letters exhibit that the data is significantly difference between treatments based on Duncan's test ($\alpha = 0.05$)

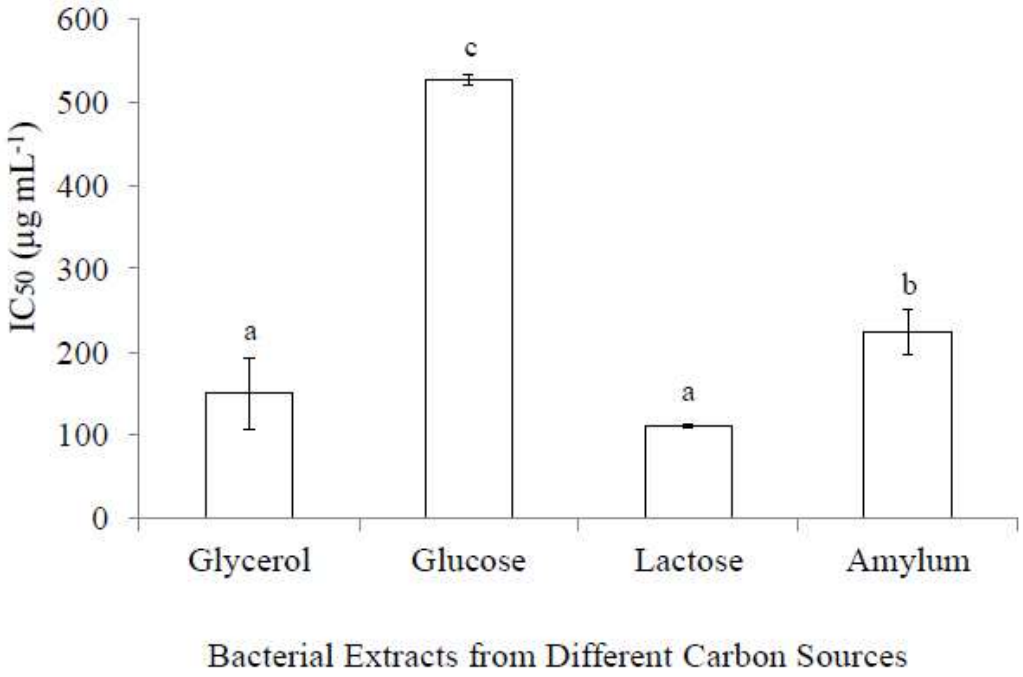

Fig. 5. Anticancer activity of HAA 01 extract cultured in different carbon sources against MOLT4 cells. Error bars represent standard deviations. Different letters exhibit that the data is significantly difference between treatments based on Duncan's test ($\alpha = 0.05$)

Biochemical Identity of the Most Potential Isolate

HAA 01 strain showed some of the biochemical profiles and carbon source utilization in AP0I CHB strips. Based on its characteristics, HAA 01 strain was closely related to *Bacillus subtilis* with 99.8 % similarity.

Discussion

Sponge associated bacteria can produce bioactive compounds that expected to have some of the medicinal property, especially anticancer activity. The bacterial crude extract used in this study may contain the variety

of compounds. In this study, 6 bacterial metabolites showed different anticancer activities, specific for each cancer cell lines. Supporting those results, reduction of cell density, morphological changes including cell shrinkage and formation of the apoptotic body of cancer cells lines, particularly CEM6, were found. As it might be due to the inhibitory effect of bacterial extract treatment.

Apoptosis is an important mechanism to remove unnecessary and unwanted cells (Korbakis and Scorilas, 2012). Induction of apoptosis is a predominant mechanistic approach to target cancer in cancer treatment. To confirm the anticancer activity of four extracts with the best IC_{50} value, apoptosis analysis was conducted on MOLT4 cells. Indeed, treatment with HAA 01 extract showed the strongest effect in causing apoptosis of MOLT4 cells. We found that MOLT4 cells treated with HAA 01 extract have fewer viable cells (70.5 %) compared than untreated cells (98.7%). Our data indicate the future application of HAA 01 extract in treating MOLT4 cells. MOLT4 cells belong to an acute lymphoblastic leukemia, which is cancer that starts from the early (immature) stage of lymphocytes in the bone marrow. Without treatment, most people with acute leukemia would live only a few months while most of the people with chronic leukemia can live for many years (ACS, 2017). Thus, further analysis regarding the specific target of HAA 01 extract toward MOLT4 cells proliferation should be conducted.

Based on our cell cycle analysis, HAA 01 likely has an anti proliferative property through cell cycle arrest, as the sub-G1 phase of MOLT4 cells was accumulated following extract treatment. It is worth noting that the HAA 01 extract substantially shows the cytotoxic effect against MOLT4 cells via cell cycle arrest as well as apoptosis induction. Those results are in agreement with several previous studies. In instance, bacterial extract from sponge associated bacteria decreases the number of MOLT4 cells in S phase, G0/G1 and G2/M, while most of the cells are accumulated the sub-G1 phase (Karwati *et al*., 2015). Exposure of MOLT4 cells to benfluron resulted in the accumulation of the cells primarily in late S and G2/M phases (Seifratová *et al*., 2015). Similarly, doxorubicin, an anticancer drug, can activate p53-DNA binding for the G1 block (Amoutzias *et al*., 2016).

In the previous study, bacterial isolate HAA 01 have been detected to have both *PKS* and *NRPS* genes (Safari *et al*., 2016), the genes that have an important role in the bioactive compound synthesis (Amoutzias *et al*., 2016). Selection of suitable medium for bacterial cultivation is very important to produce bioactive compounds, especially anticancer compounds. In this study, utilization of glucose as carbon source downregulated the expression of both *NRPS* and *PKS* genes, compared to glycerol. On the other hand, lactose induced the expression of *NRPS* genes in a significantly higher level compared to that glycerol. The synthesis of secondary metabolites may correlate with the preferential use of medium carbon source. Some of the carbon sources can inhibit the transcription of specific genes encoding bioactive compounds. These mechanisms are renowned as carbon catabolite repression (Ruiz *et al*., 2010). Our study suggests that glycerol, lactose and amylum could be the preferred carbon source in HAA 01 strain to optimize the expression of *NRPS* and *PKS* genes. While glucose repressed the expression of both genes. In addition to that gene expression results, the bacterial extract derived from glucose-containing culture exhibited depletion of its anticancer activity against MOLT4 cells.

The carbon source may activate the anticancer properties or, on the contrarily, inhibit the formation of anticancer compounds. Although glucose is a preferable carbon source for primary metabolism (Ilić *et al*., 2010), however, the glucose may inhibit secondary metabolites synthetic pathway in microorganisms (Ruiz *et al*., 2010). In our experiment, lactose and glycerol showed a great potential as a carbon source for the production of anticancer compounds in this experiment, instead. Similarly, previous study reported that lactose, glycerol, amylum were found to be suitable carbon source to produce secondary metabolites, including elaiophylin and hexaene H-85 (Ilić *et al*., 2010), AK-111-81 macrolide antibiotic (Gesheva *et al*., 2005), prodigiosin (Elkenawy *et al*., 2017), rapamycin (Kim *et al*., 2014), MSW2000 antibiotic (El-Naggar *et al*., 2003). On the other side, glucose, which the most simple of medium carbon source, is affecting the synthesis of some bacterial metabolites. Similar to the results in this study, glucose depresses prodigiosin production in *Serratia marcescens* by influencing the activity of Glucose Dehydrogenase (GDH) activity (Fender *et al*., 2012) and decreasing the pH (Sole *et al*., 1997). The presence of glucose also inhibited the synthesis of actinorhodin in *Streptomyces lividans*. Glucose repressed the specific gene for stimulation of secondary metabolites production, *afsR2* (Kim *et al*., 2001). Therefore, the utilization of medium carbon source is the main factor required for optimizing the production of bacterial bioactive compounds.

Our study indicates a unique correlation between *NRPS-PKS* gene expression in different carbon sources toward its respective anticancer activity. A high anticancer activity is in line with the high expression of *NRPS-PKS* genes, exhibited by the extract from glycerol, lactose and amylum-culture. Conversely, the low expression of *NRPS-PKS* in glucose correlates with the low anticancer activity of the extract. These data strongly suggest that *PKS-NRPS* genes are likely involved in the synthesis of anticancer compounds, particularly of that HAA 01 isolate. HAA 01 isolate has both *PKS* and

NRPS genes, thus this isolate may potentially synthesize anticancer compounds from the hybrid *NRPS-PKS* genes. Some of the bioactive compounds have been reported to be synthesized from such hybrid *NRPS-PKS* mechanism, including bleomycin (Mizuno *et al*., 2013), leinamycin (Tang *et al*., 2006) and curacin A (Chang *et al*., 2004). Further research is needed to verify the role of these genes in the biosynthesis of anticancer compounds.

Based on the phenotypic identification, HAA 01 strain was identified as *Bacillus subtilis* with high similarity. This result is consistent with the previous genotypic identification based on 16S rRNA that was conducted by Wahyudi (2016). The previous study reported that marine *Bacillus* can produce some of the bioactive compounds including bacillistatin, mixirin and halobacillin that potential as anticancer (Mondol *et al*., 2013). Moreover, these studies suggest the future application of marine bacterial extract as a new anticancer drug.

Conclusion

From our study, it is worth noting that secondary metabolites from sponge associated bacteria could be potential as anticancer based on the inhibition of cancer cell proliferation, initiation of apoptosis and cell cycle arrest of that cancer cell lines. Our study indicates that anticancer activity of HAA 01 extract is highly correlated with *NRPS-PKS* gene expression which is likely controled by the type of carbon source.

Acknowledgement

The authors thank to Ministry of Finance (LPDP), Ministry of Research, Technology and Higher Education, Indonesia and MEXT, Japan, that have been promoted this work.

Funding Information

This project was granted by Beasiswa Pendidikan Indonesia, LPDP (Indonesia Endowment Fund for Education), ministry of finance [PRJ-1580/LPDP/2015]; Penelitian Unggulan Perguruan Tinggi, The Ministry of Research, Technology and Higher Education [079/SP2H/LT/DRPM/II/2016] to ATW; and the Japan re-inventory project "TWINCLE program", MEXT, Japan to JAP. Therefore, we thank for all the supports.

Author's Contributions

Jepri Agung Priyanto: Has contributed in a whole experiment, data analysis and publication.

Rika Indri Astuti: Has assisted in the experimental design, results confirmation, interpretation of data and revising the article critically.

Jun Nomura: Has contributed in providing materials for anticancer analysis, interpretation of data and paper preparation.

Aris Tri Wahyudi: Has lead the project, connected the research outcome and involved in paper preparation.

Ethics

This article is authentic from authors works. The corresponding author ensures that all of the other authors have read and recognized the manuscript.

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