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Identification of *Streptomyces* spp. isolated from air samples and its cytotoxicity of anti-MRSA bioactive compounds

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ABSTRACT

Rapid emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has made the search of a novel bioactive compound a necessity. In this study, two selected strains of actinomycetes (KB1 and KB3) were isolated from air samples from Aonang, Krabi province, southern Thailand and identified as *Streptomyces* spp., using 16S rDNA sequence analysis. The active molecules in crude extract of fermented broth from strain KB1 exhibited anti-MRSA activity. Partial characterization of the active molecules (heat labile) showed that it would be of protein nature. The identification of its cytotoxicity is an important first step in improving active metabolites safely use for human health. In this study, the cytotoxicity (IC_{50}) of active molecules against Vero cells was found to be 12.5 μg protein/ml when compared with ellipticine (0.48 μg/ml). The results indicated that active molecules in bioactive compounds had the mild cytotoxic activity against Vero cells. Future works will investigate the mechanism of action, confirm the *in vivo* cytotoxicity, along with study the pharmacodynamics and pharmacokinetics of this compound, to develop bioactive compounds from the natural resources.

1. Introduction

The drug resistance problem demands to discover new antimicrobial substances effective against resistant pathogenic bacteria. Today, methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as one of the important resistant pathogenic bacteria in community-acquired and hospital-acquired infections. It is possibly the greatest concern of all health-care-associated pathogen due to its ability to cause a wide variety of life-threatening infections (Schito, 2006; Montazeri et al., 2013). The screening of novel antibiotics from natural resources have been interested. One of the many natural resources is bacterial strain in the group of streptomycetes. Streptomycetes were reported to be flora of the most natural environments such as soil, freshwater, sediment and air (Lertcanawanichakul, 2015). Accounting to 70–80% of actinomycetes screened from soil, relevant secondary metabolites available commercially (Baltz, 2008). The compounds produced by actinomycetes which have diverse clinical effects and important applications in human medicine (Watve et al., 2001). It has been estimated that approximately one-third of the much more of naturally occurring antibiotics have been recovered from actinomycetes (Takizawa et al., 1993). The majority of these compounds demonstrate one or more bioactivities, many of them developed into drugs for treatment of a wide range of diseases in human, veterinary and agriculture sectors. (Bernan et al., 1997). Searching for novel actinomycetes constitutes an essential component in natural product-based drug discovery). The strains of genus *Streptomyces* are superior to other actinomycetes in their ability to produce large number and varieties of secondary metabolites (Usha et al., 2015).

Streptomyces are prokaryotic gram-positive, aerobic bacteria which produce an extensive branch mycelia that rarely fragment (Goodfellow et al., 1988). They are known to produce varieties of antimicrobial substances (Bull and Stach, 2007). Discovery of new antibiotics produced by actinomycetes, *Streptomyces*, still continues as an essential component in natural product-based drug discovery. Exploring new habitats is one of the most promising ways to isolate new strains of actinomycetes endowed with antimicrobial activity (Zitouni et al., 2005; Khanna et al., 2011; Wadetwar and Patil, 2013). This has resulted in isolation of a promising new strain of *Streptomyces* species from air samples collected at the Krabi province, Thailand. Herein, report the isolation and molecular identification of a *Streptomyces* strains endowed with anti-methicillin *Staphylococcus aureus* (anti-MRSA) activity. Anti-MRSA and cytotoxic activities of the bioactive compounds in culture (fermented) broth are also described.

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2. Material and methods

2.1. Isolation of streptomyces strains

The organisms were isolated from air samples collected from Aonang, Krabi province, southern Thailand, using Biosampler apparatus, Microflow 90, with the flow rate 100 l/min for 30 min according to the manufacture's instruction. The air samples were collected at one time in summer season, sunny day. The phenotypic characteristics were determined by the methods as previously described (Shirling and Gottlieb, 1966; Arai et al., 1975). The colonies of actinomycetes were recognized according to their macroscopic and microscopic characteristics (optical microscopy and Gram stain), embedded colony and Gram positive branching bacteria were selected. Primary screening of anti-MRSA activity was performed on yeast extract-malt extract agar (YMA) plates (Anansiriwattana et al., 2006) (Himedia) or half-formula Luria-Bertani agar (LB/2) (2.5 g/l yeast extract, 5 g/l tryptone, 5 g/l NaCl, 15 g/l agar) plates by cross streak method (Lertcanawanichakul and Sawangnop, 2008). Presence of reduced growth of test bacteria near the growth of actinomycetes was considered as positive for antagonistic activity. Secondary screening of the strains was examined by the agar well diffusion method (Barry and Thornsberry, 1985) against methicillin-resistant *S. aureus* 142 (MRSA 142). Pure culture of the strains were maintained on LB (Oxoid) agar slant and conserved at 4 °C for short periods and at −20 °C in glycerol stock (20%, v/v) for a longer period. The organisms were identified on the basis of 16S rDNA studies. The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strains KB1 and KB3 were deposited in url of NCBI website under GenBank data.

2.2. Sequence analysis

2.2.1. PCR amplification of 16S rDNA

DNA templates for PCR amplification were prepared by using "Genomic DNA mini kit (Blood/culture cell)" (Geneaid Biotech Ltd., Taiwan). DNA coding for 16S rRNA regions was amplified by means of PCR with Taq polymerase, as previously described (Kawasaki et al., 1993; Yamada et al., 2000; Katsura et al., 2001). A PCR productfor sequencing 16S rDNA regions was prepared by using the following two primers, 20F (5′-GAG TTT GAT CCT GGC TCA G-3′, positions 9–27 on 16S rDNA by the *Escherichia coli* numbering system) and 1500R (5′-GTT ACC TTG TTA CGA CTT-3′, position 1509–1492 on 16S rDNA by the *E. coli* numbering system) (Brosius et al., 1981). The PCR amplification was carried out with DNA Engine Dyad Thermal Cycler (Bio-Rad Laboratories). One hundred μl of a reaction mixture contained 15–20 ng of template DNA, 2.0 μmoles each of the two primers, 2.5 units of Taq polymerase, 2.0 mM $MgCl_2$, 0.2 mM dNTP and 10 μl of 10xTaq buffer, pH 8.8, containing $(NH_4)_2SO_4$, which was comprised of 750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20. The PCR amplification was programmed to carry out an initial denaturation step at 94 °C for 3 min, 25 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and elongation at 72 °C for 2 min, followed by a final amplification step at 72 °C for 3 min. The PCR product was analyzed by 0.8% (w/v) agarose gel electrophoresis and purified with a QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany). The purified PCR product was stored at −20 °C for further step.

2.2.2. Direct sequencing of 16S rDNA

Direct sequencing of the single-banded and purified PCR products (ca. 1500 bases, on 16S rDNA by the *E. coli* numbering system was carried out. Sequencing of the purified PCR products was carried out with an ABI PRISM BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (version 3.1, Applied Biosystems, Foster City, California, USA). The primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 800R (5′-TAC CAG GGT ATC TAA TCC-3′) for partial sequencing, and additional 518F (5′-CCA GCA GCC GCG GTA ATA CG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) for full length sequencing were used for sequencing of 16S rDNA. Ten μl of a sequencing reaction mixture contained 5–20 ng of template DNAs, 2.0 μl of BigDye™ terminator ready reaction mixture, 5–20 ng of DNA template, 1.6 pmole of sequencing primer, 1.5 μl of 5xBigDyeTM sequencing buffer and deionized water. The PCR reactions were carried out as follows: an initial denaturation step at 96 °C for 30 s, 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and elongation at 60 °C for 4 min. Eighty μl of freshly prepared ethanol/acetate solution was added to the sequencing reaction mixture in 1.5 ml microcentrifuge tube, and mixed well with a brief vortex. The mixture was left to stand at room temperature for 15 min and centrifuged at the maximum speed or 14,500 rpm for 20 min at room temperature. The ethanol solution was immediately removed carefully from the tube with an aspirator equipped with a fine tip. The resulting DNA pellets were washed by adding 250 μl of 70% ethanol to the tube, and vortexed briefly. The precipitated DNA was collected by centrifugation for 5 min at the maximum speed. The remaining ethanol was carefully removed from the tube with an aspirator equipped with a fine tip. The DNA obtained was dried in a heat box at 90 °C for 1 min, and the dried DNA was stored at either 4 °C or −20 °C. The DNA pellets were suspended in 20 μl of a terminator sequencing reagent, mixed on a vortex and spun down. The double-stranded DNA was completely separated by heating at 95 °C for 2 min, and immediately placed on ice, until ready to load on instrument. The DNA sequencing was performed on an ABI Prism 3730XL DNA Sequence (Applied Biosystems, Foster City, California, USA).

The identification of phylogenetic neighbors was initially carried out by the BLASTN (Altschul et al., 1997) program against the database containing type strains with validly published prokaryotic names (Kim et al., 2012). The top thirty sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using global alignment algorithm (Thompson et al., 1997), which was implemented at the EzTaxon-e server (http://eztaxone ezbiocloud.net).

2.3. Phylogenetic analysis

The DNA sequences determined and obtained from databases were aligned with a program CLUSTAL X (version 1.8) (Thompson et al., 1997) in BioEdit Program. (Hall, 1999). Alignment gaps and unidentified bases were eliminated. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). Phylogenetic trees of 16S rRNA genes were constructed by the neighbor-joining method described by Saitou and Nei (1987). The robustness for individual branches was estimated by 1000 replications bootstrapping (Felsenstein, 1985) with the program MEGA Version 5.2.1 (Tamura et al., 2011). 16S rDNA sequence of *Kitasatospora setae* KM-6054T (APO10968) was used as an out-group. Gaps and ambiguous nucleotides were eliminated from the calculations.

2.4. Antimicrobial activity

2.4.1. By cylinder agar method (agar plug method)

Firstly, the actinomycetes isolates were sown in scratches tightened in two agar media YMA, and LB/2 to show which medium stimulates maximum antimicrobial activity. After incubation for 7 days at 30 °C, agar cylinders near colony not exceed 0.5 mm were then taken with hollow punch and placed on Mueller Hinton (MH) agar plates, previously seeded with the test microorganisms (10^5–10^6 CFU/ml) (Jiménez-Esquilín and Roane, 2005; Elleuch et al., 2010). The Plates were kept at 4 °C for 2 h, and then incubated at 37 °C and observed for antibiosis, inhibition zone, after 24–48 h. The bacteria used as targets were *S. aureus* TISTR 517 and MRSA 142 (clinical isolate).

2.4.2. By agar well diffusion

A loopful of each selected strain was inoculated into a 25 × 125-mm screw-capped test tube containing 10 ml of YM broth or LB/2 broth and incubated on a rotary shaker at 200 rpm, 30 °C for 7 days. The culture broth of each selected strain was collected by centrifugation at 12,000 rpm, 4 °C for 15 min. Then, it was filtered through 0.45 µm pore size membrane, cell free supernatant (CFS), transferred to sterile microcentrifuge tube. The CFS was used to test the anti-MRSA activity by agar well diffusion method (Barry and Thornsberry, 1985).

S. aureus TISTR517 or MRSA142 was cultivated on MH (HiMedia) agar slants at 37 °C for 24 h. The broth cultures of test bacteria were swabbed on sterile MH agar plates followed by punching wells of 6 mm diameter using sterile cork borer. 100 µl of CFS was transferred into labeled wells and the plates were incubated at 37 °C for 24 h. The plates were observed for zone of inhibition formed, if any, and measured using a ruler (Magaldi et al., 2004; Valgas et al., 2007). The experiment was carried in replicate and the average value ± SD was recorded.

2.5. Partial characterization of the anti-MRSA products

The sensitivity to heat was examined by heating the CFS of each selected strain to 60 °C for 30 min, 100 °C for 30 min and 121 °C for 15 min then the treated CFS was tested against *S. aureus* TISTR517 and MRSA142 to determine its anti-*Staphylococcus* activity or anti-MRSA activity by the agar well diffusion method. After incubation for 24 h at 37 °C, the inhibition zone was measured.

2.6. Bradford protein assay

Bradford reagent concentrate was purchased from Bio-Rad. The concentrated reagent was diluted 1:4 and filter through Whatman no. 1 paper just before use. The spectrophotometer was warmed up before use. The 20 µl of CFS that collected from selected strain was mixed thoroughly in working solution (1 ml), stand still at room temperature for 5 min, measured the absorbance at 595 nm. The bovine serum albumin (BSA) at the concentration of 0.2–1 mg/mL was used as standard for setting calibrating curve and determined amounts of protein from the curve (Bradford, 1976). The concentration of original samples was determined from the amount protein, volume/sample, and dilution factor, if any.

2.7. Cytotoxicity against primate cell line (vero cells)

The GFP-expressing Vero cell line was generated in-house by stably transfecting the African green monkey kidney cell line (Vero, ATCC CCL-81), with pEGFP-N1 plasmid (Clontech). The cell line is maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 1 mM sodium pyruvate, 1.5 g/l sodium bicarbonate and 0.8 mg/ml geneticin, at 37 °C in a humidified incubator with 5% CO_2. The assay was carried out by adding 45 µl of cell suspension at 3.3×10^4 cells/ml to each well of 384-well plates containing 5 µl of test compounds were previously diluted with 0.5% DMSO, and then incubated for 4 days in 37 °C incubator with 5% CO_2. Fluorescence signals are measured by using SpectraMax M5 microplate reader (Molecular Devices, USA) in the bottom reading mode with excitation and emission wavelength of 485 and 535 nm, respectively. Fluorescence signal at day 4 is subtracted with background fluorescence at day 0. The percentage of cytotoxicity is calculated by the following equation, where FUT and FUC represent the fluorescence units of cells treated with test compound and untreated cells, respectively: $\%\ \text{cytotoxicity} = [1\text{-}(\text{FUT/FUC})] \times 100$, where FUT and FUC are the mean fluorescent intensity from treated and untreated conditions respectively. IC_{50} values are derived from dose-response curves, using 6 concentrations of 3-fold serially diluted samples, by the SOFTMax Prosoftware. Ellipticine and 0.5% DMSO or phosphate buffer saline (PBS) are used as a positive and a negative control (Laungsuwon and Chulalaksananukul, 2013).

3. Results and discussion

3.1. Isolation and selection of the actinomycetes strains

The embedded colonies on media agar plate showed the fungal-like formation (aerial mycelium, substrate mycelium) were selected for Gram staining. Two selected isolates are Gram positive. The isolates showing different morphological characteristics were named as KB1 and KB3. Actinomycetes have evolved as a group with greatest genomic and metabolic diversity (Shuvankar et al., 2012). Atinomycetes account of the earth's surface and represent an attractive source for isolation of novel microorganisms and production of potent bioactive compounds (secondary metabolites). Herein, a total of two selected actinomycetes strains were isolated from air samples collected at Aonang, Krabi province, Thailand based on their morphological and cultural characteristics.

The cross-streaking is an easy and relatively rapid method for screening cultures in search for new antibiotics and thus establish a spectrum of inhibiting properties of any bacterium, mold, or Actinobacteria which will grow discretely on an agar plate. As a result, the inhibitory activity on tested bacteria by cross streak method or agar plug method as preliminary screening for antimicrobial activity is seen as better than those obtained by agar well diffusion method (Lertcanawanichakul and Sawangnop, 2008). However, in bacteriocin or secondary metabolites investigations, the cross streak method should be controlled by the agar well diffusion method, it could be caused of the major drawback of the ‘cross streak method’ was difficulty in obtaining quantitative data, since the margins of the zone of inhibition were usually very fuzzy and indistinct. On the secondary screening of the strains by agar well diffusion, the selected strains exhibited antibacterial activity against *S. aureus* TISTR 517 and MRSA142. These finding indicates that both of them showed effective against *S. aureus* TISTR 517.

Novel antibiotics against drug resistant bacteria are urgently needed. Microbial natural products have been one of the major sources of novel drugs. In this study showed that exploring new habitats is one of the most promising ways to isolate new strains of actinomycetes endowed with antimicrobial activity, especially anti-MRSA activity. In several previous studies, the *Streptomyces antibioticus* and *Streptomyces flaveolus* were antibacterial substances producing strains, actinomycins and polycyclic ethers, respectively. These antibacterial substances were inhibited Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. (Kelner, 1949; Shomura et al., 1970; Fawaz and Jones, 1988; Jones, 2000). It was also reported that *Streptomyces psammoticus* produced polyketide antibiotics effective against MRSA (Sujatha et al., 2005).

In this study, selected actinomycetes isolates named KB1 and KB3. The isolates were Gram positive and formed tough and filamentous colonies that were hard to pick from the culture media as a characteristic of actinomycetes. Strain KB1 produced colored pigments (white) after growing on YMA plate for at least 7 days. Whereas, strain KB3 produced the orange-brown pigments, secreted into the culture media as shown in Fig. 1. On primary screening, the strains showed the anti-*S. aureus* TISTR 517 and MRSA142.

3.2. 16S rDNA sequence and phylogenetic analyses

On the basis of morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including phylogenetic analyses of 16S rDNA sequences. The selected strains (KB1 and KB3) were identified as *Streptomyces*. Spore chain morphology of the selected strains were rectiflexibiles or spiral. They showed the same chemotaxonomic pattern which were similar to the member of the genus *Streptomyces* (Holt, 1989).

According to 16S rDNA gene amplification, the resulted sequence was subjected to similarity searches against public databases to infer

Fig. 1. The colony morphology of the strain KB1 and KB3 on yeast extract malt-extract agar (YMA) plate.

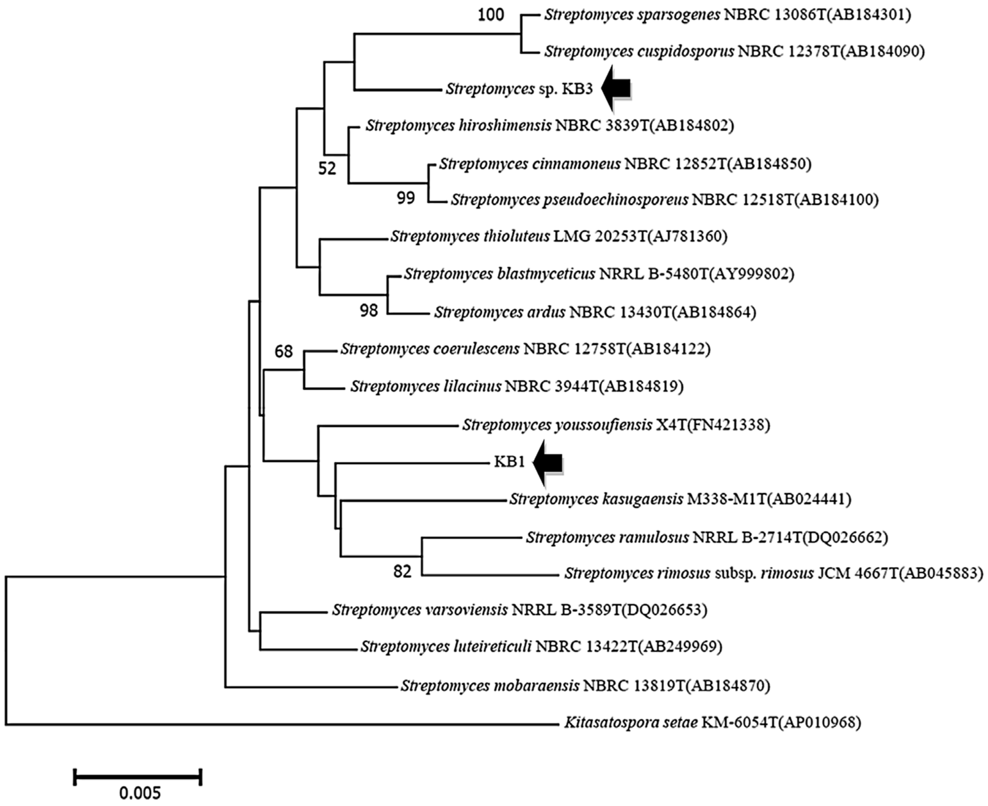

Fig. 2. The phylogenetic analysis showed the position of the strain KB1 and KB3 with other *Streptomyces* based on 16S rDNA gene. Phylogenetic tree based on neighbor-joining analysis of 1000 resampled data. Number at nodes indicates the percent level of bootstrap support. The scale bar represents 0.005 substitutions per nucleotide position.

Table 1
The effect of temperature on anti-MRSA activity of active molecules in bioactive compounds from strain KB1.

Strains	Zone of inhibition (mm)			
	25 °C 30 min	60 °C 30 min	100 °C 15 min	121 °C 15 min
MRSA142	22.50 ± 0.71	16.00 ± 0.00	12.50 ± 0.71	6.00 ± 0.00
S. aureus TISTR 517	19.00 ± 0.00	12.50 ± 0.71	10.00 ± 0.00	6.00 ± 0.00

possible phylogenetic relationship of the selected strains, KB1 and KB3. The phylogenetic tree (Fig. 2) from representative strains of the related species indicated that the both of which should be placed in the genus *Streptomyces* comparing with some of the type strains validly described, and *Kitasatospora setae* KM-6054T (APO10968) was selected as an outgroup (Fig. 2). In the comparison of 16S rDNA gene sequences, the strain KB1 was mostly related with *Streptomyces varsoviensis* NRRL B-3589T (98.98%). Whereas, strain KB3 was mostly related with *Streptomyces hiroshimensis* NBRC 3839T (99.51%). However, both of which may be classified as a newly strain when identified by 16S rRNA gene sequencing. The sequences have been deposited in the GenBank under the accession: KF939581.1, GI: 566568264 for strain KB1. For strain KB3, its sequence was deposited under the accession: KF939582.1, GI: 566568265. The cultures were deposited in Thailand Institute of Science and Technology Research (TISTR) under number 2304 and TISTR 2305 for strain KB1 and KB3, respectively.

The review articles related about *Streptomyces* and antibiotics produced by *Streptomyces* have been reported (Annaliesa and Elizabeth, 2001; de Lima et al., 2012). For example, natamycin, also known as pimaricin, is a polyene macrolide antibiotic, produced in submerged cultures of certain *Streptomyces* strains such as *Streptomyces natalensis*, *Streptomyces chattanoogensis*, and *Streptomyces lydicus* (de Lima et al., 2012). Expected newly strains, KB1 and KB3, exhibited good

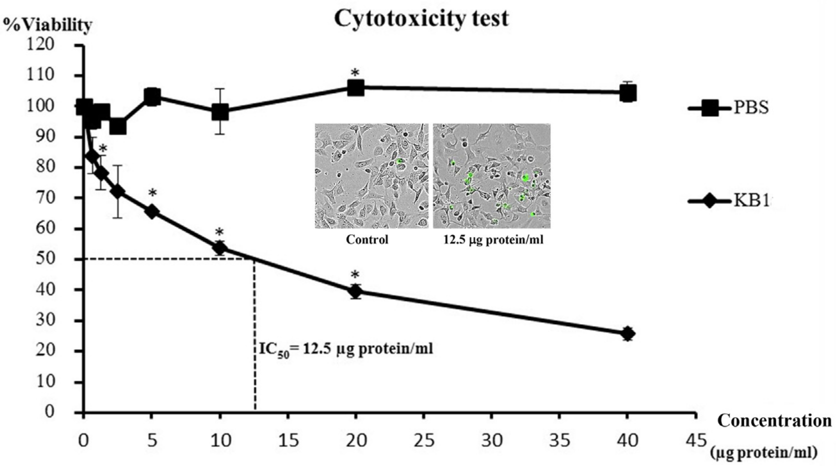

Fig. 3. Effect of active molecules in bioactive compounds from strain KB1 on Vero cells (IC$_{50}$ value = 12.5 µg protein/ml). Phosphate buffer saline (PBS)-treated cells are controls.

antimicrobial activity against *S. aureus* TISTR 517 and MRSA strains. Interestingly, crude extract from the fermented broth from strain KB1 did show the apoptosis activity when analyzed by cell cycle arrest (data not shown). Such, the fermented broth from strain KB1 was selected to use for testing the stability of anti-MRSA compounds and cytotoxicity on Vero cells.

3.3. *Effect of temperature on anti-MRSA activity*

The effect of temperature parameter on anti-MRSA activity was studied for strain KB1. The anti-*S. aureus* activity or anti-MRSA activity was decreased after placing at temperature more than 100 °C. However, at temperature 25 °C was still had anti-MRSA activity of bioactive compound (20–28 mm) as tabulated in Table 1.

After placing the bioactive compounds at different temperature, then anti-MRSA activity was investigated. The anti-MRSA activity was lost when placed the bioactive compound at temperature more than 100 °C for 30 min. It implied that the effective molecule may be the protein because it cannot resist to high temperature. Further studies should be needed to confirm results by means of NATIVE or sodium dodecyl sulphate polyacylamide gel electrophoresis (SDS-PAGE) analyzing for anti-MRSA activity (Somsap et al., 2016).

Because culture is growing at a constant growth rate and no obvious stress is being imposed on the mycelium, particularly at pH 7.0, our data provide strong support that protein secretion is not occurring through some cultural perturbation and may be linked to antibiotic production as an important element of secondary metabolism in strain KB1. Further work is now in progress to identify the reasons and regulatory factors that operate secretory functions in strain KB1 during the phase of secondary metabolism, also the effective of media components and pH parameter were studied.

3.4. *Cytotoxicity assay*

Vero cells are derived from African Monkey kidney, it might be served as a good candidate to represent non-cancerous cells at the cytotoxicity level. Moreover, the screening for a certain drug on Vero cells have been reported by many researchers. This study was found that the bioactive compounds in fermented broth from strain KB1, contained an active molecules at a concentration of 40.0 µg protein/ml. It was used to analyze cytotoxicity by means of GFP-based fluorescent detection (Hunt et al., 1999). Positive control (ellipticine) or active molecules were two-fold serially prepared and tested. The cytotoxicity property after the treatments with various concentration of positive control and bioactive compounds observed in Vero cells. The tested concentrations from 0.13 µg/ml to 4.0 µg/ml for ellipticine. Whereas, the final concentrations of active molecules varied from 0.62 µg protein/ml to 40.0 µg protein/ml. The cytotoxicity was observed at IC$_{50}$ value of 12.5 µg protein/ml for active molecules (Fig. 3), whereas for ellipticine was observed at IC$_{50}$ value of 0.48 µg/ml.

The active molecules and reference standards showed cytotoxicity against Vero cells at different final concentrations. Unfortunately, the results indicated that active molecules was higher cytotoxicity effective against Vero cells when compared with ellipticine tested. Although, ellipticine has been observed to induce remission of tumour growth by inhibited the enzyme topoisomerase II via intercalative binding to DNA (Auclair, 1987), but it is not used for medical purposes due to its high toxicity; side effects include nausea and vomiting, hypertension, cramp, pronounced fatigue, mouth dryness, and mycosis of the tongue and oesophagus (Paoletti et al., 1980). It is meaning that ellipticine is classified as a prodrug (Stiborová et al., 2012).

By the ways, brine shrimp lethality bioassay has been ever used to tested preliminarily assess the cytotoxicity of the active molecules. It did not show cytotoxicity against brine shrimp (Chawawisit et al., 2015). The cytotoxicity shows the variations depending on the chemical nature of the compound and the route of administration to the experimental animal (Sudha Kesavan et al., 2011; Roy et al., 2006). Therefore, *in vivo* cytotoxicity study in rat along with the pharmacodynamics and pharmacokinetics will be performed in the next time, including the mechanisms of action have to concerned for further study in another mammalian cell lines.

4. Conclusion

Isolation of two selected actinomycetes, KB1 and KB3, from air samples collected by Microflow 90 apparatus. Both of them showed anti-MRSA activity. The selected actinomycetes have been identified as *Streptomyces* sp. based on morphological, and 16s rRNA gene sequencing. However, the anti-MRSA activity was lost when the fermented broth was placed at temperatures over 100 °C. It implied that the effective molecule may be the protein. Fortunately, the bioactive compounds remain active at temperature 25 °C on the left for 30 min. Unfortunately, these findings found that the bioactive compounds in fermented broth might be toxic to normal cell, Vero cell line. It should be tested the cytotoxicity to other cell lines for confirmation the data. By the way, it suggests that the strain could be clinically important need to be investigated further for the antibiotic resistant bacteria, investigate the mechanism of action, confirm the *in vivo* cytotoxicity in rat, along with study the pharmacodynamics and pharmacokinetics of this compound.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101236.

Conflicts of interest

The authors declare that there is no conflict of interest.

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