Tropical Journal of Pharmaceutical Research November 2017; 16 (11): 2727-2734
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i11.21

Original Research Article

Investigation of antibacterial and anti-cancer activities of Streptomyces sp SRF1 culture filtrate

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Sent for review: 7 December 2016 *Revised accepted: 5 October 2017*

Abstract

Purpose: *To evaluate the antibacterial activity and cytotoxic effects of Streptomyces sp. SRF1 culture filtrate extract against breast cancer cell line.*
Methods: *The activity of the extract against Gram-positive and Gram-negative bacteria was initially screened by an agar-well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were measured by broth microdilution method. Time-kill assays were also performed, and extract-induced morphological and ultrastructural changes to bacterial cells were investigated. Sulforhodamine B (SRB) assay was performed to determine the cytotoxicity of the extract against the human breast cancer cell line, MCF-7.*
Results: *Antibacterial activity by the extract was detected against four strains of Gram-positive pathogens including one strain of methicillin-susceptible Staphylococcus aureus (MSSA) and 3 strains of methicillin-resistant Staphylococcus aureus (MRSA) - with low MIC and MBC values. This activity was bactericidal after 6 h exposure. Morphological alterations were detected on the cell surface of both MSSA and MRSA. The extract also inhibited MCF-7 cell growth with half-maximal concentration (IC_{50}) of 211.67 ± 33.95 µg/mL in 72 h.*
Conclusions: *Streptomyces sp. SRF1 culture filtrate extract exhibits potent antibacterial and anticancer activities and thus, represents a potential source of antibacterial and anticancer drugs.*

Keywords: *Antibacterial activity, Anti-breast cancer, Staphylococcus aureus, Streptomyces sp. SRF1*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Soil microorganisms are an interesting source of new bioactive compounds. Compounds produced by such organisms can be developed and used in both agriculture and medicine [1-2]. Actinomycetes are soil bacteria known to produce various novel bioactive compounds. They are present in nature in a wide range of habitats, exhibit considerable species diversity, and considerable diversity in terms of their morphology, physiology, and biochemical activities [3]. Bioactive compounds produced by actinomycetes can potentially be developed as novel therapeutic agents.

It was recently reported that 70 % of all antibiotics in use were derived from actinomycetes bacteria [2], 75 % of these from the genus *Streptomyces* [4-6]. Antibiotics derived

from *Streptomyces* have antibacterial, antifungal, antioxidant, and antitumor activities [7-11]. For example, Lee *et al* [12] reported that the soil *Streptomyces* sp. AMLK-135 had activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The mangrove soil *Streptomyces pluripotens* MUSC 137 is reported to have cytotoxic activity against human cancer cell lines [11]. Ethyl acetate extract from rock soil *Streptomyces* spp. ERI-3 has been shown to inhibit the growth of *Staphylococcus aureus*, *S. epidermidis* and *Candida albicans* [7]. Moreover, other soil *Streptomyces* spp. such as *S. antibioticus*, *S. flaveolus* and *S. psammoticus* have been shown to inhibit MRSA [13].

Previous studies in our laboratory have shown that *Streptomyces* sp. SRF1, an isolate from rice field soil in northeast Thailand, has *in vitro* antifungal activity against some economically important plant pathogens [14]. Crude mycelial and culture filtrate extracts of isolate SRF1 also inhibit growth of the foodborne pathogen *Bacillus cereus*, inducing its cells to become elongated and abnormal in shape, and to lyse [15]. However, no information is available on the activity of isolate SRF1 against other medically important pathogens or against human cancer cell lines. The aim of the present study, therefore, was to elucidate the effects of isolate SRF1 culture filtrate extract against human pathogenic bacteria by agar well diffusion, by the microdilution and time-kill methods, and by scanning electron microscopy (SEM). Cytotoxic activity of the extract was also investigated against human breast cancer cell line MCF-7 in this study using the SRB method.

EXPERIMENTAL

Preparation of culture filtrate extract

Seven–day old mycelial discs of *Streptomyces* sp. isolate SRF 1 on half PDA were cut and placed into 50 mL of Arginine glycerol mineral salt (AGMS) medium before being incubated at 37 ˚C with shaking at 250 rpm. After 14 days, the culture filtrate was collected and filtered through Whatman filter paper no. 1 before extraction with ethyl acetate by a liquid-liquid extraction method. The collected ethyl acetate layer was then evaporated under reduced pressure. The 50 mg of yellow brown gum extract obtained was then re-suspended in 0.1 mL of methanol and adjusted to 50 mg/mL final concentration using distilled water, before being tested for activity against 16 pathogenic bacteria (Table 1) and the MCF-7 breast cancer cell line.

Preliminary screening for antibacterial activity

The activity of the culture filtrate extract was preliminary screened against 16 pathogenic bacteria by agar well diffusion method as described by Sangdee *et al* [16]. In brief, the 16 bacterial strains were cultured in 5 mL Mueller Hinton broth (MHB) and then they were incubated at 37 °C with shaking at 250 rpm for 3 h. These were then adjusted to the 0.5 McFarland standard before being swabbed to the surface of Mueller Hinton agar (MHA). Next, wells were cut using a sterile cork borer, and 0.1 mL of culture filtrate extract was added to each well (final concentration of 50 mg/mL per well). After 16 – 18 h incubation (37 °C), the inhibition zones in each plate were measured. The antibiotics tetracycline and ciprofloxacin were used as reference standards at concentrations of 250 µg/mL.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Next, the antibacterial activity of the culture filtrate was determined quantitatively. Because zones of inhibition were only detected against *Staphylococcus aureus* (MSSA) DMST 2933, MRSA DMST 4738, MRSA DMST 20651 and MRSA DMST 20654, MIC and MBC assays were only performed with these strains. MIC and MBC values of the extracts were determined using the microdilution method. Briefly, a -3 - h culture of test bacteria was adjusted to $4 \times 10^6 – 5 \times 10^6$) CFU/mL, and 10 µL aliquots of this were added to the wells of a 96 - well polystyrene tray. Ninety microliter volumes of a diluted culture filtrate extract (25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195 and 0.09 mg/mL) in MHB were then added. A growth control well, and an uninoculated control well were included in each plate. Tetracycline at a concentration of 250 µg/mL was used as a positive control. Wells with the lowest concentration of extract that remained clear after incubation were recorded as the MIC. MBC values were determined by identifying the lowest concentration of extract capable of killing the test bacterium.

Time-kill assay

The bactericidal or bacteriostatic activity of the culture filtrate extract against MRSA DMST 20651 and MSSA DMST 2933 were performed by time – kill assays using a method modified from Thammawat *et al* [17]. Briefly, bacterial cultures in mid - logarithmic growth phase were standardized to the 0.5 McFarland standard and

then they were inoculated into several tubes of MHB containing an equal volume of various concentrations of extract (0.78, 0.39 and 0.195 mg/mL) so as to give final concentrations of 0.39 mg/mL (2x MIC), 0.195 mg/mL (1x MIC), and 0.09 mg/mL (0.5x MIC) extract, respectively. Test tubes of MHB without extract and MHB with 1x MIC tetracycline were included in each experiment. The final bacterial cell density was approximately 4 – 5 x 10^5 CFU/mL. All tubes were incubated at 37 °C. At 0, 2, 4, 6 and 24 h, time intervals, a small volume of bacterial suspension was removed, diluted, and spread onto the surface of MHA plates so that viable counts could be performed. The number of bacteria remaining in each sample was then plotted over time to determine the rate of killing. A three $\log_{10}$ reduction in bacterial numbers was considered to indicate bactericidal activity.

Investigation of the effect of culture filtrate extract on bacterial cell morphology

The effects of the culture filtrate extract on the surface characteristics and shape of MRSA DMST 20651 and MSSA DMST 2933 were investigated using a scanning electron microscope. Bacterial cells were incubated at 37 °C for 12 h with shaking at 250 rpm, then harvested, resuspended in 0.5 mL MHB, and inoculated into 0.5 mL of 1 x MIC levels of the test extract. Samples were incubated at 37 °C for 6 h with shaking at 250 rpm. Next, the bacterial cell pellets were collected and washed twice with 1 mL phosphate - buffered saline (PBS) before being fixed with 2.5 % glutaraldehyde in 5 % sucrose overnight at 4 °C. Next, each cell pellet was dehydrated in an alcohol series. A total volume of 15 μL of each sample was then applied to membranes. Dried samples were sputter - coated with gold and examined.

Evaluation of anti-breast cancer cell line activity

The anti-breast cancer cell line MCF-7 activity of the culture filtrate extract was elucidated using a SRB assay using a method modified from Buranrat *et al* [18] and Sangdee *et al* [19]. Briefly, MCF-7 cells were cultured in a 96 - well plate for 24 h. After that, the cells were exposed to the culture filtrate extract at concentrations of 1 to 500 μg/mL for 24 - 72 h. Next, the culture medium was removed before being fixed with 10 % cold trichloroacetic acid for 1 h at 4 °C. Then, the plates were washed five times with deionized water, air – dried, and 0.4 % SRB in 1 % acetic acid was added for 30 min at room temperature. Next, the plates were washed three times with-1 % acetic acid to remove any excess dye. After that, 200 μL of 10 mM Tris-base solution was added to solubilize the SRB dye within the cells. Next, the absorbance was measured at 540 nm using a micro-plate reader.

Statistical analysis

The data were analyzed using *t*-test. Significant differences between treatments and control group were considered significant at $p < 0.05$. Statistical analyses was performed using SPSS statistical software (SPSS 14, SPSS Inc, IL, USA).

RESULTS

Antibacterial activity of culture filtrate extract

The activity of *Streptomyces* sp. SRF1 culture filtrate extract was investigated against 16 strains of human pathogenic bacteria. The extract inhibited the growth of 4 strains of Gram positive staphylococci, with large inhibition zone diameters compared to the control antibiotics tetracycline and ciprofloxacin. None of the test strains of Gram negative bacteria were affected (Table 1). Interestingly, MRSA DMST 20651 was resistant to ciprofloxacin and tetracycline at the tested concentration, but sensitive to the culture filtrate extract. MRSA DMST 20654 was also resistant to ciprofloxacin, but sensitive to the extract. Based on these results, only the 4 sensitive staphylococcal strains were selected for further antibacterial testing.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs and MBCs of the extract were determined by a broth microdilution method. The results show that the MICs and MBCs of the extract were quite low. MIC and MBC values of 0.195 and 0.390 mg/mL, respectively, were determined for all four test strains, MSSA DMST 2933, MRSA DMST 4738, MRSA DMST 20651, and MRSA DMST 20654 (Table 1).

Antibacterial activity

Time-kill assays were performed to determine if the activity of *Streptomyces* sp. SRF1 culture filtrate extract is bacteriostatic or bactericidal. Bactericidal activity was defined as a ≥ 3 $\log_{10}$ decrease in CFU/mL of the bacterial population compared with the untreated control, while bacteriostatic activity was defined as a < 3 $\log_{10}$ decrease in CFU/mL. The time kill profiles

Table 1: Activity of *Streptomyces* sp. SRF1 culture filtrate extract against 16 strains of bacterial pathogen using the antibiotics ciprofloxacin and tetracycline as reference standards

Test bacterium	Zone of inhibition (mm)			Broth microdilution method	
	Culture filtrate extract (5 mg)	***Ciprofloxacin (25 μg)***	***Tetracycline (25 μg)***	***MIC (mg/mL)***	***MBC (mg/mL)***
S. aureus (MSSA) DMST 2933	36x36	25x25	32x32	0.195	0.390
*S. aureus (*MRSA) DMST 20651	37x37	0	0	0.195	0.390
MRSA DMST 4738	35x35	26x26	33x33	0.195	0.390
MRSA DMST 20654	31x31	0	22x22	0.195	0.390
V. cholerae (O1) DMST 9700	0	38x38	30x30	ND	ND
E. coli (EIEC) DMST 30545	0	20x20	0	ND	ND
E. coli O157:H7 DMST 12743	0	30x30	22x22	ND	ND
Sh. flexneri DMST 4423	0	33x33	0	ND	ND
Sh. flexneri DMST 17569	0	35x35	0	ND	ND
S. Typhi DMST 22842	0	33x33	0	ND	ND
S. Typhi DMST 16122	0	34x34	28x28	ND	ND
Sh. dysenteriae DMST 15110	0	33x33	20x20	ND	ND
S. Typhimurium ATCC 14028	0	31x31	26x26	ND	ND
Ps. aeruginosa ATCC 27853	0	25x25	0	ND	ND
Ps. aeruginosa	0	25x25	0	ND	ND
K. pneumonia	0	30x30	25x25	ND	ND

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; ND, not determined

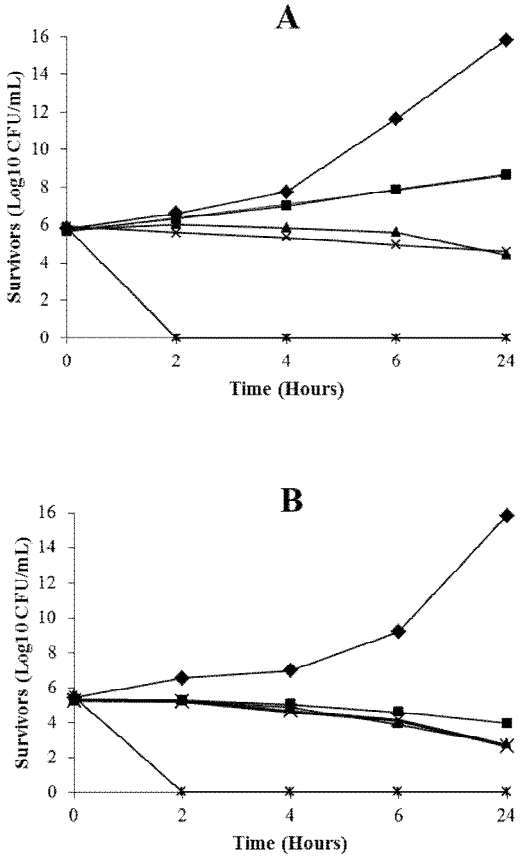

Figure 1: *In vitro* bactericidal activity of *Streptomyces* sp. SRF1 culture filtrate extract against MRSA DMST 20651 (A) and MSSA DMST 2933 (B) at concentrations of 0.09 mg/mL (0.5 x MIC), 0.195 mg/mL (1 x MIC), and 0.39 mg/mL (2 x MIC) compared to an untreated control and tetracycline (0.195 mg/mL). ♦: untreated control; ■: 0.5 x MIC; ▲: 1 x MIC; ×: 2 x MIC; ∗: tetracycline

showed that the culture filtrate extract and standard tetracycline antibiotic were bactericidal against both MSSA DMST 2933 and MRSA DMST 20651. At 0.5 x MIC (0.09 mg/mL), the extract was able to reduce viable cell counts of both *S. aureus* strains by more than 3 $\log_{10}$ during within 6 h. At concentrations of 1 x MIC (0.195 mg/mL) and 2 x MIC (0.39 mg/mL) also resulted in bactericidal activity against both *S. aureus* strains by 6 h, with lower viable cell counts than at 0.5 x MIC. After 24 h incubation with 1 x MIC (0.195 mg/mL) and 2 x MIC (0.39 mg/mL) levels of the extract, some of the treated bacteria remained viable but a 10 $\log_{10}$ CFU/mL reduction in viability of both MSSA and MRSA had occurred (Figure 1). These results indicate that the extract exhibited concentration- and time-dependent bactericidal activity.

Morphological features

The effect of *Streptomyces* sp. SRF1 culture filtrate extract on the morphology of MSSA DMST 2933 and MRSA DMST 20651 cells was investigated by SEM. Treatment of *S. aureus* with 1 x MIC levels of extract (0.195 mg/mL) induced morphological alterations in the bacterial cells. MSSA DMST 2933 bacterial cells became bloated and crushed after 4 h treatment. Also, some bacterial cells showed cavities and others were lysed (Figure 2B). Similar changes were observed following tetracycline treatment (concentration equal to 1 x MIC of the extract) (Figure 2C). Untreated control cells did not show any alterations in cell morphology (Figure 2A). In MRSA DMST 20651 treated with extract for 4 h,

some cells were bloated and crushed, cell surfaces were disrupted and broken, and some cells were lysed (Figure 2E). Similar morphological alterations occurred when MRSA was treated with tetracycline at a concentration of 1 x MIC (Figure 2F). Untreated MRSA cells did not appear damaged (Figure 2D).

Anti-breast cancer cell activity

The effects of various concentrations (1 - 500 μg/mL) of *Streptomyces* sp. SRF1 culture filtrate extract on the cell viability of breast cancer cell line MCF-7 were investigated using the SRB assay. High concentrations of extract reduced the viability of MCF-7 cells more than lower concentrations. Incubation time also directly affected cell viability compared with the control (Figure 3). The IC_{50} of the extract against MCF-7 cells was 471.33 ± 52.82, 391.67 ± 20.58 and 211.67 ± 33.95 μg/mL for 24, 48 and 72 h, respectively. The highest Emax value, 71.53 ± 5.13, was recorded at an exposure time of 72 h, followed by 48.91 ± 6.10 and 57.53 ± 5.41 for 24 and 48 h, respectively. These results indicate that the extract exhibited concentration - and time - dependent cytotoxic activity against the MCF-7 cell line.

DISCUSSION

Bacteria in the *Streptomyces* genus are well known to have bioactive secondary metabolite producing activity, these products widely used by the pharmaceutical industry for their antimicrobial, antiviral [20] and anticancer activities [9]. In previous studies, we demonstrated that *Streptomyces* sp. SRF1, isolated from a rice field, inhibits the growth of phytopathogenic fungi and the food-borne bacterial pathogen *Bacillus cereus* under *in vitro* conditions [14,15]. In the present work, the activity of this culture filtrate extract was investigated against other important bacterial strains and the breast cancer cell line MCF-7. The results demonstrate that the extract has activity against 4 strains of *Staphylococcus aureus*, but no activity against any of the test strains of Gram negative bacteria. These results may reflect the mode of antibacterial action of the extract. Because Gram negative and Gram positive bacteria differ in their cell wall structure, they have different characteristics in terms of available target site and molecule adsorption and uptake [21].

The results show that the pathogens MSSA DMST 2933, MRSA DMST 4738, MRSA DMST 20651, and MRSA DMST 20654 were quite sensitive to the culture filtrate extract of *Streptomyces* sp. SRF1. Of these four strains, MRSA DMST 20651 had the highest resistance to ciprofloxacin and tetracycline antibiotics. Next, time - kill assays were performed with the culture filtrate extract on MRSA DMST 20651 and MSSA DMST 2933. These results indicate the culture filtrate extract is bactericidal, reducing viable cell counts of the tested *S. aureus* strains by more than 3 $\log_{10}$ in 6 h. Larger reductions in viable cell count (more than 10 $\log_{10}$) were observed when the extract concentration and exposure time were increased (Figure 1A and 1B). These results indicate that the culture filtrate extract kills *S. aureus* in both a concentration - and time - dependent manner. This result correlate with the

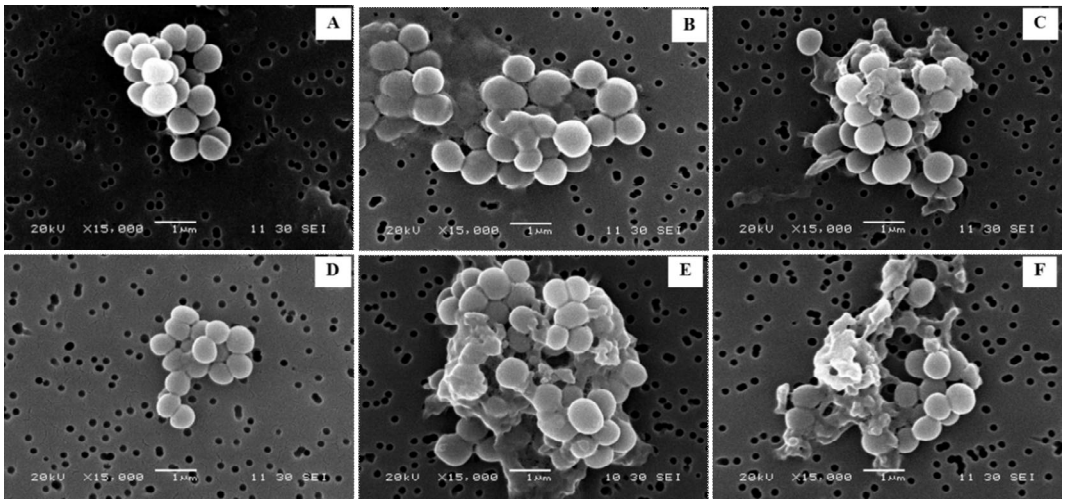

Figure 2: Scanning electron micrographs of *S. aureus* (MSSA) DMST 2933 (A - C) and *S. aureus* (MRSA) DMST 20651 (D - F) after treatment with *Streptomyces* sp. SRF1 culture filtrate extract at 1 x MIC (0.195 mg/mL) (B and E), and the antibiotic tetracycline at 0.195 mg/mL (C and F) for 4 h as compared with untreated controls (A and D)

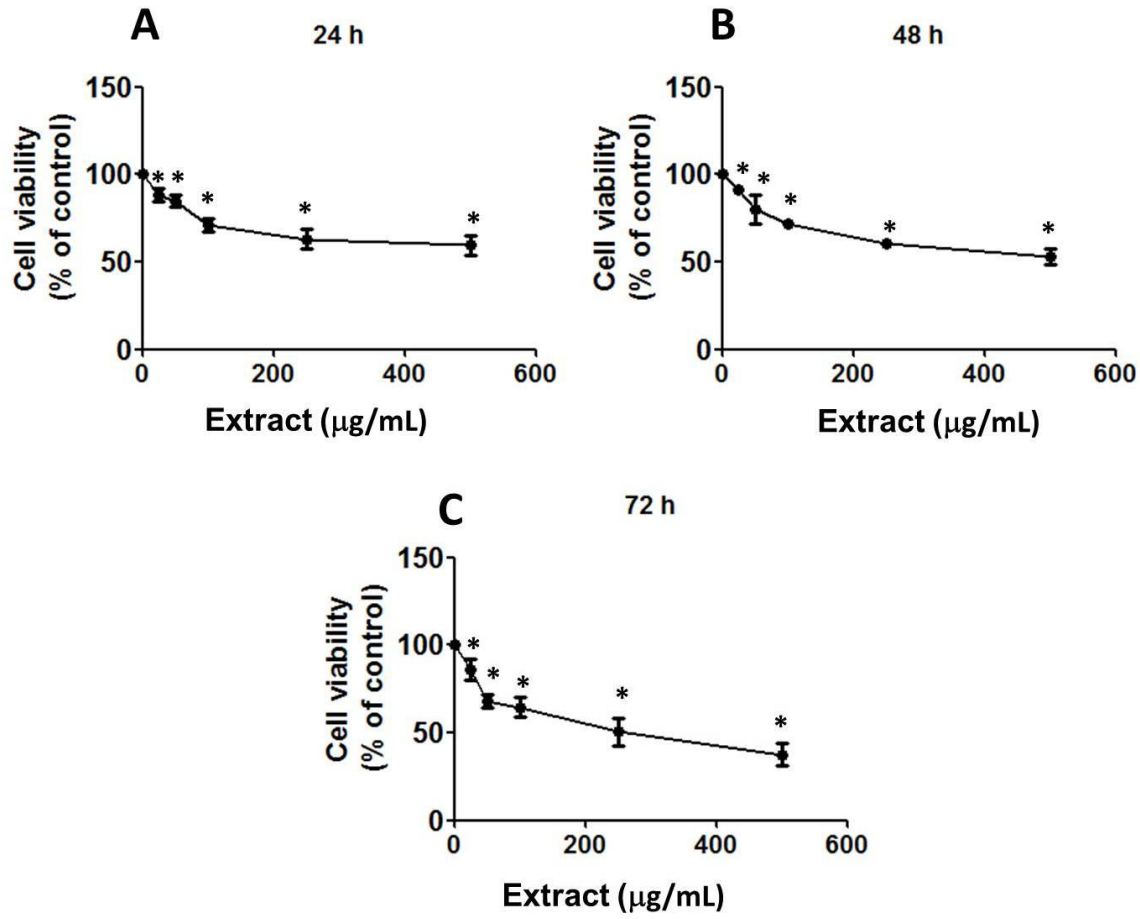

Figure 3: Effects of *Streptomyces* sp. SRF1 culture filtrate extract on the proliferation of breast cancer cell line MCF-7. MCF-7 cells were treated with various concentrations of 1 – 500 µg/mL for 24 - 72 h. The MCF-7 cell proliferation was measured by the SRB assay after treatment. The experiment was done in three independent experiments. The results are presented as percentage of control, and represent mean ± SEM values. *$p < 0.05$ vs. untreated control groups

opinion of Iniyan *et al* [22], who demonstrated that the phenolic compound T1 isolated from a marine-derived actinomycete *Micromonospora* sp. ICN36 inhibited the growth of MRSA and MSSA at low concentrations (MIC values of 0.5 and 0.25 µg/mL, respectively). Moreover, the 4 x MIC of the phenolic compound T1 showed bactericidal activity against MRSA after 4 h exposure [22].

To determine if the culture filtrate extract might exert its antibacterial activity by targeting the cell wall, we examined the effects of the extract on *Staphylococcus aureus* using SEM. At 1 x MIC, the extract altered the surface appearance and morphology of both MSSA and MRSA cells. Observed changes included cell bloating, a crushed appearance, cell surface disruption, and lysis. These cell alteration effects may be due to bioactive constituents in the culture filtrate extract disrupting peptidoglycan biosynthesis [23,24]. Many bioactive compounds produced by *Streptomyces* spp., for example phenolic compounds and antibiotics, have been shown to alter cell surface appearance. Previous observations include wrinkling of the cell surface and cell shrinkage [22,24,25].

Results from the anti-breast cancer assay clearly indicate that *Streptomyces* sp. SRF1 culture filtrate extract has concentration- and time-dependent cytotoxic activity against the MCF-7 cell line. This effect may be due to bioactive compounds present in the extract. Bioactive compounds isolated previously from *Streptomyces* spp. have been reported to inhibit the activation of survival signaling pathways in cancer cells [26,27]. Ser *et al* [11] demonstrated that a fermentation extract derived from *S. pluripotens* MUSC 137 had both significant antioxidant activity and significant cytotoxic activity. Several cancer cell lines were shown to be susceptible including MCF-7. *S. parvus* extract has also been shown to be active against breast cancer cell lines [28]. Isolation and structure elucidation of the compounds

responsible for the bioactivities of *Streptomyces* sp. SRF1 culture filtrate extract are now underway in our laboratory.

CONCLUSION

The findings of this work show that *Streptomyces* sp. SRF1 culture filtrate extract has bactericidal activity against staphylococci bacteria including methicillin-resistant *S. aureus*. Furthermore, the extract also exerts activity against MCF-7 breast cancer cell line. Thus, *Streptomyces* sp. SRF1 culture filtrate extract, after appropriate further investigation, may yield antibacterial and anticancer drug candidates. Further studies will now focus on isolating the compounds responsible for the observed bioactivities and elucidating their structures.

DECLARATIONS

Acknowledgement

The authors are grateful to Mahasarakham University Faculty of Medicine for equipment support and language-editing assistance, and Mahasarakham University Faculty of Science for provision of laboratory space and equipment. B. Buranrat is grateful to Thailand Research Fund (grant no. MRG6080071). P. Seephonkai is grateful to the Center of Excellence for Innovation in Chemistry for support (no. PERCH-CIC).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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