International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.06 pp 709-717, 2016

Antibacterial activity, time-kill profile and morphological effects of *Streptomyces* sp. SRF1 extracts against the foodborne pathogen *Bacillus cereus*

Sangdee K[1], Pimta J[2], Seephonkai P[3], Sangdee A[2,4*]

[1]Microbiology Group, Biomedical Sciences Research Unit, Faculty of Medicine, Mahasarakham University, Muang District, Maha Sarakham 44000, Thailand
[2]Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham 44150, Thailand
[3]Nano Technology Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham 4415, Thailand and Center of Excellence for Innovation in Chemistry (PERCH-CIC)
[4]Microbiology and Applied Microbiology Research Unit, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham 44150, Thailand

Abstract : The soil bacterium *Streptomyces* sp. SRF1 is known to have activity against fungal pathogens of plants, but limited information is available on its medical and pharmaceutical potential. The objective of this study was to investigate the effects of the mycelial and culture filtrate extract of *Streptomyces* sp. SRF1 on growth of *Bacillus cereus*. Both extracts were active against *B. cereus* with low MIC and MBC values. The MIC values of the mycelial and culture filtrate extract were 0.39 mg/ml and 0.0195 mg/ml, respectively. The MBC values of the mycelial extract and culture filtrate extract were 0.39 mg/ml and 0.3125 mg/ml, respectively. The optimal concentration and duration of treatment to achieve *B. cereus* cell death were also determined. Concentrations of 4xMBC and 8xMBC of the extracts completely killed the bacterial cells after 2 hours exposure. Concentrations of 1xMBC and 2xMBC, by contrast, did not completely kill the bacterial cells. However, bacterial cell numbers were reduced after 24 hours treatment with both extracts compared with those of the 0.5xMBC treated and control groups. When *B. cereus* cells were examined by light microscopy after 2 hours treatment with the extracts, they were elongated to multiple times their original size and had a collapsed appearance. This study indicates that the isolate *Streptomyces* sp. SRF1 represents a potentially new source of antibiotics that could be developed as therapeutic agents.
Keywords: antibacterial, *Bacillus cereus*, *Streptomyces* sp., time-kill.

Introduction

Bacillus cereus is an aerobic, Gram-positive, rod-shaped and spore-forming bacterial species that is widely distributed in soil, marine environments, vegetables, the intestinal tracts of invertebrate hosts, and human skin. This organism is a relatively common cause of food poisoning and opportunistic infections in susceptible hosts.[1] Moreover, *B. cereus* causes necrotic enteritis, liver failure, bacteraemia, endocarditis, meningitis, pneumonia, endophthalmitis and skin lesions.[2] Recently, difficulties have been reported treating *B. cereus* infections, with some strains resistant to the antibiotics erythromycin, tetracyclines, carbapenem,

clindamycin, penicillin, cephalosporins and many of the other cell-wall active antibiotics including ampicillin, cephalothin, and methicillin.[1, 2, 3] Therefore, many scientists are screening *Streptomyces*, *Bacillus*, *Penicillium* and other species for new microbial products that could be developed as novel therapeutic agents.[4]

Antibiotic-producers in the *Streptomyces* genus are Gram-positive bacteria that grow in various environments especially in soil, plants, and fresh and marine water.[5, 6] The shape of *Streptomyces* cells resembles filamentous fungi and their hypha can differentiate to chains of spores.[7] Many species of the genus *Streptomyces* are known as produce important bioactive secondary metabolites with interesting activities including antibacterial, antifungal[8], antiviral[9], antiparasitic, antitumor and immunosuppressive activities.[7] For example, the mannopeptimycins are cyclic glycopeptide antibiotics produced by *S. hygroscopicus* LL-AC94 that have moderate to good antibacterial activity against methicillin-resistant staphylococci and vancomycin-resistant enterococci.[10] Also, the verticillate *Streptomyces* sp. (MSU-2110) produces coronamycin complex peptide antibiotics with activity against the human fungal pathogen *Cryptococcus neoformans* and the malarial parasitoid *Plasmodium falciparum*.[11] Some strains of soil *Streptomyces* have been reported to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*.[5] Moreover, the cyclic depsipeptides hormaomycin B and hormaomycin C, isolated from a marine mudflat-derived *Streptomyces* sp., have activity against some Gram-positive and Gram-negative bacterial pathogens.[12] These antagonistic activities indicate that actinomycetes in the genus *Streptomyces* are a useful source of bioactive compounds with potential therapeutic activity against pathogenic bacteria and fungi.

In a previous study, we isolated several *Streptomyces* sp. from various plant rhizosphere soils and found the soil isolate *Streptomyces* sp. SRF1 to have activity against 6 plant pathogenic fungi including *Colletotrichum gloeosporioides, C. capsici, Biporalis maydis, Pyricularia* sp., *Fusarium* sp., and *Curvularia* sp. by the dual culture method.[13] However, literature describing the activity of isolate SRF1 against medically important pathogens is limited. Therefore, the aim of the present study was to extract bioactive compounds from the mycelial and culture filtrate of *Streptomyces* sp. SRF 1 and to determine the *in vitro* activity of the extracts against the foodborne bacterial pathogen *B. cereus*. Activity was also assessed by the time-kill method and by examining the effects of the extracts on bacterial morphology.

Materials and Methods

Bacterial Culture

Streptomyces sp. isolate SRF 1 was cultured in half PDA at 37˚C for 7 days. The mycelial discs were cut and placed into 50 mL of Arginine glycerol mineral salt (AGMS) medium. The seed culture was incubated at 37˚C with shaking at 250 rpm/min for 14 days. The culture filtrate was collected by centrifugation at 4,000 rpm for 10 min at 4˚C and filtered (Whatman No. 1) before extraction. Mycelia were oven-dried at 40˚C, powdered, and then used for extraction.

The bacterial culture of *Bacillus cereus* ATCC 11778 was obtained from the Culture Collection for Medical Microorganisms, Department of Medical Sciences, Thailand. This was used as the antimicrobial test organism. The bacteria were maintained on Mueller Hinton agar at 37˚C.

Mycelium and Culture Filtrate Extraction

The mycelial extraction was performed by resuspending 1 gram of the dried mycelium powder obtained from *Streptomyces* sp. isolate SRF 1 with 50% ethanol (100 mg/mL) and then sonicating this with a High Intensity Ultrasonic Processor (Model VCX750, Newtown, CT, USA). This step was performed on ice for a total of 5 min in 10-s bursts with 2-s gaps for cooling. The sonicated solution was centrifuged at 4000 rpm for 5 min before being filtered through a 0.2-mm filter. The sonicated solution was then tested for antibacterial activity.

The culture filtrate extraction was performed by liquid-liquid extraction using ethyl acetate as the organic solvent. The collected ethyl acetate layer was evaporated under reduced pressure to give a yellow brown gum. The 20 mg of extract obtained was then resuspended in 0.1 mL of methanol and adjusted to a final volume of 1 mL using distilled water (20 mg/mL). This was also tested for antibacterial activity.

Antibacterial Activity

The mycelial and culture filtrate extracts were tested for antibacterial activity by the agar well diffusion method. *Bacillus cereus* ATCC 11778 was cultured in Mueller Hinton broth (MHB) and incubated at 37°C with shaking (200 rpm/min) for 3 h before being adjusted to the 0.5 McFarland standard. The standardized bacterial suspension was then applied to the surface of Mueller Hinton agar (MHA) plates using a sterile cotton swab, with the inoculum streaked in four directions to achieve complete coverage. Wells were cut in the agar using a sterile cork borer. The mycelial extract, culture filtrate extract, and controls were added into each well (0.1 mL per well). After incubation of the plates at 37°C for 16–18 h, the zone of inhibition surrounding each well was measured in millimeters. Solutions of 10% (v/v) methanol and 50% (v/v) ethanol were used as negative controls for the culture filtrate extract and mycelial extract, respectively. Solutions of tetracycline and ciprofloxacin, at concentrations of 250 µg/ml, were used as positive controls.

Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs)

The minimum inhibitory concentrations of the active extracts were determined by the agar well diffusion method. The procedure was the same as above. The concentrations tested were two-fold serial dilutions starting from 100 mg/mL mycelial extract and 20 mg/mL culture filtrate extract, respectively. The MIC was defined as the lowest concentration of the extract able to inhibit bacterial growth surrounding the well. The minimum bactericidal concentrations of the active extracts were determined by the broth microdilution method. A 3-h culture of the test bacteria was adjusted to 4-5 × 10^6 CFU/mL. Polystyrene trays containing 96 wells were prepared containing 90 µL of 2-fold dilution concentrations of the extracts in MHB. Each well was then inoculated with ten microliters of the standardized bacterial suspension. A growth control well and an uninoculated control well were included in each plate. After incubation at 37°C for 16–18 h, the suspension in each well was subcultured on MHA. The MBC was defined as the lowest concentration of the extract able to kill the test bacterium.

Time-Kill Assay

Test bacteria in mid-logarithmic growth phase were adjusted to the 0.5 McFarland standard. Then, the standardized bacterial suspension was inoculated into several tubes of MHB containing various concentrations of the extracts (8xMBC, 4xMBC, 2xMBC, 1xMBC and 0.5xMBC) and a growth control tube without the extracts. The final density of bacteria was approximately 4-5x10^5 CFU/mL. These tubes were incubated at 37°C. Then, small aliquots were removed at specific time intervals of 0, 2, 4 and 24 h and diluted to obtain countable numbers of colonies by spread plate method. The number of bacteria remaining in each sample was plotted over time to determine the rate of killing. A three $\log_{10}$ reduction in bacterial counts indicates bactericidal activity. All experiments were performed in duplicate.

Morphological Effects

The effect of the extracts on bacterial cell morphology was investigated by light microscopy. *Bacillus cereus* ATCC 11778 in mid-logarithmic growth phase was adjusted to the 0.5 McFarland standard. Then, the standardized bacterial suspension was inoculated into several tubes of MHB containing 2xMBC extract, a high concentration of both extracts (100 mg/mL mycelial extract and 20 mg/mL culture filtrate extract), and a growth control tube without extract. The final density of bacteria was approximately 4-5x10^5 CFU/mL. These tubes were incubated at 37°C for 0 and 2 h. Then, the cell pellets were collected by centrifugation at 10,000 rpm for 5 min and washed three times with sterile PBS. The cell pellet was fixed with 2.5% glutaraldehyde in 5% sucrose overnight at 4°C. The bacterial cells were stained with Safranin O before examination by light microscopy.

Results and Discussion

Antibacterial Activity

Antibacterial activity of the mycelial and culture filtrate extracts of *Streptomyces* sp. SRF1 was measured using MIC and MBC assays. Both extracts inhibited the growth of *Bacillus cereus* ATCC 11778, with large inhibition zone diameters compared to the control antibiotics tetracycline and ciprofloxacin (Fig. 1 and 2).

Interestingly, the MIC and MBC values of both extracts were quite low. The MIC and MBC values of the mycelial extract were 0.39 and 0.39 mg/mL, while the MIC and MBC values of the culture filtrate were 0.0195 and 0.3125 mg/mL, respectively. This compares favorably with a study by Cwala *et al*.[14], who reported that a crude extract of *Streptomyces* sp. isolate TR007 had an MIC of 2.5 mg/mL and MBC of 10 mg/mL against *B. subtilis*. Also, Sharmin *et al*.[15] reported that a crude ethyl acetate from *Streptomyces* sp. isolate ANTS-1 had MIC and MBC values ranging from 1-2 and 1-4 µg/mL, respectively. Moreover, a decursin substance from *Streptomyces* sp. GMT-8 isolated from *Zingiber officinale* Rosc. could inhibit the growth of Gram-positive bacteria including *B. cereus* with an MIC of 256 µg/mL.[16] The lower the MIC and MBC value an agent has, the more potent it is as an antibacterial agent.[17]

Fig. 1: Zones of *B. cereus* ATCC 11778 inhibition caused by 100 mg/mL (1), 50 mg/mL (2), 25 mg/mL (3), 12.5 mg/mL (4), 6.25 mg/mL (5), 3.125 mg/mL (6), 1.56 mg/mL (7), 0.78 mg/mL (8), and 0.39 mg/mL mycelial extract (9), and 250 µg/mL tetracycline (Tet), 250 µg /mL ciprofloxacin (Cip), and 50% ethanol (C).

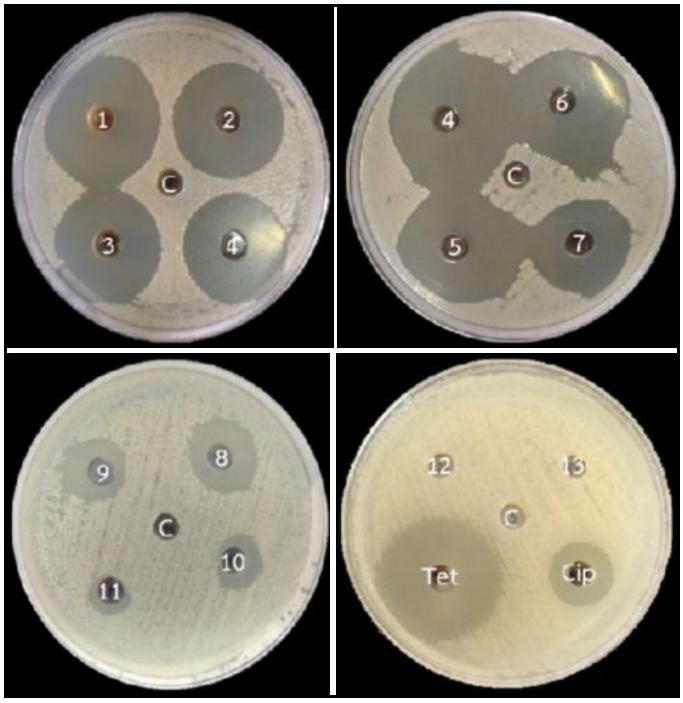

Fig. 2: Zones of *B. cereus* ATCC 11778 inhibition by 20 mg/mL (1), 10 mg/mL (2), 5 mg/mL (3), 2.5 mg/mL (4), 1.25 mg/mL (5), 0.625 mg/mL (6), 0.3125 mg/mL (7), 0.156 mg/mL (8), 0.078 mg/mL (9), 0.039 mg/mL (10), and 0.0195 mg/mL culture filtrate extract (11), and 250 µg/mL tetracycline (Tet), 250 µg /mL ciprofloxacin (Cip), and 10% methanol (C).

Bactericidal or Bacteriostatic Activity

The time-kill assay was used to determine whether the extracts were bactericidal or bacteriostatic. In this study, we investigated this by testing a range of concentrations above and below the MBC. The assessment was performed with *B. cereus* ATCC 11778, and cells were incubated in the presence of increasing concentrations of both extracts. Samples were taken at regular time intervals during exposure and transferred to plates of MHA medium to determine viable cell counts. The mycelial extract at concentrations of 4xMBC (1.56 mg/mL) and 8xMBC (3.12 mg/mL) showed the most effective killing, with bacterial cell counts decreasing from 5 log CFU/mL at 2 h to below detectable levels after 4 h. Treatment with 1xMBC (0.39 mg/mL) and 2xMBC (0.78 mg/mL) mycelial extract for 24 h reduced viable cell counts to 0.71 log CFU/mL and 2.19 log CFU/mL, respectively. Lastly, bacteria treated with 0.5xMBC (0.195 mg/mL) were able to survive and increase in number (Fig. 3). Similar findings were obtained with the culture filtrate extract. At both 4xMBC (1.25 mg/mL) and 8xMBC (2.50 mg/mL), the culture filtrate extract reduced bacterial cell density from 5 log CFU/mL to below detectable levels within just 2 h. After 24 h treatment with 1xMBC (0.31 mg/mL) and 2xMBC (0.63 mg/mL) culture filtrate extract, viable cell counts decreased to 2.07 log CFU/mL and 1.91 log CFU/mL, respectively. Treatment with 0.5xMBC (0.16 mg/mL) culture filtrate extract for 24 h caused a mean reduction of 0.31 log CFU/mL at 24 h compared with the initial inoculum (Fig. 3). These results indicate that the death rate and maximum reduction in viable cell count are dependent on extract concentrations, and that both extracts are bactericidal against *Bacillus cereus* ATCC 11778 at concentrations of 4xMBC and above. These results correlate with those of Ultee *et al*.[18], who reported that carvacrol is bactericidal against *B. cereus*, causing a more than 10^4-fold reduction in viable cell count compared with the initial inoculum. Our results are also in accord with the Messick *et al*. [19] definition of bactericidal activity, which states that bactericidal activity is a decrease of 3 log cfu/mL or more in the initial inoculum.

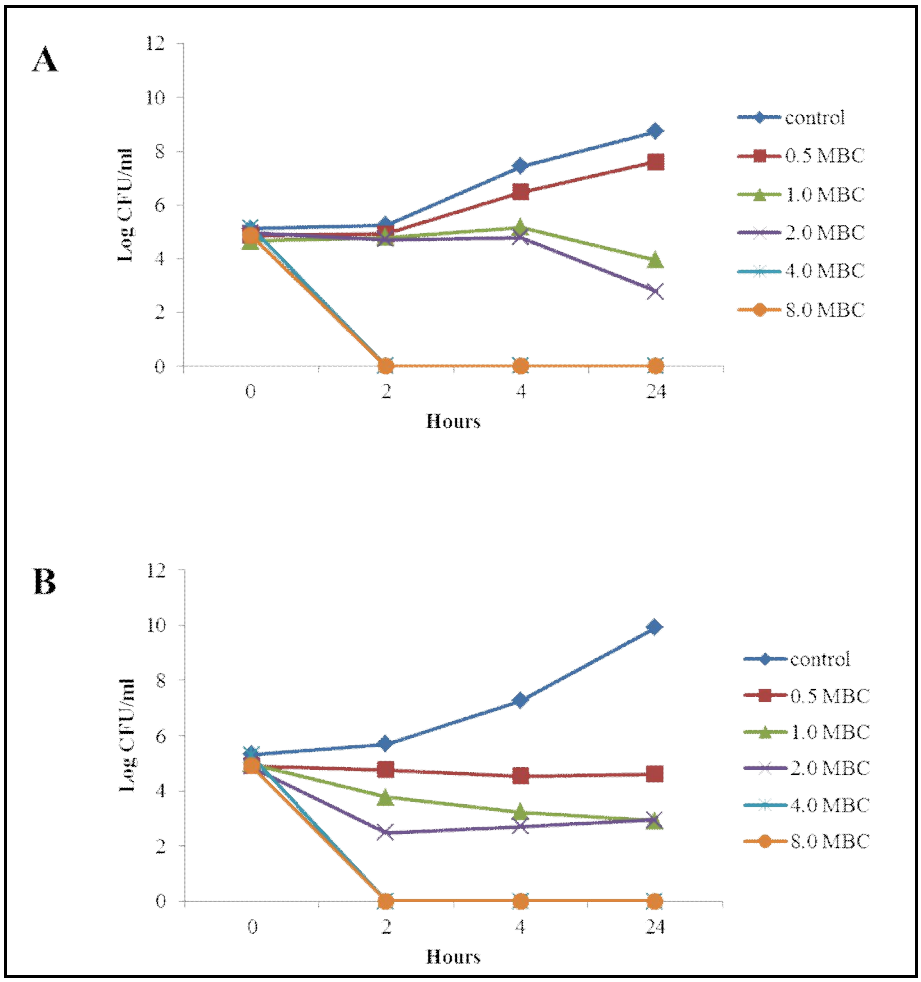

Fig. 3: Time-kill curves of *B. cereus* ATCC 11778 treated with mycelial extract (A) and culture filtrate extract (B) at the different concentrations.

Morphological Effects

The effects of 2xMBC mycelial (100 mg/mL) and culture filtrate (20 mg/mL) extracts on the morphology of *B. cereus* ATCC 11778 were determined at 0 and 2 h. At 0 h, untreated cells and bacterial cells exposed to the extracts had a normal rod shape when examined by light microscopy. In bacteria treated with 2xMBC (100 mg/mL) of the mycelial extract for 2 h, some cells had an abnormal rod shape and others were lysed (Fig. 4). This finding correlates with the results of Chahardehi *et al*.[20], who found that ethyl acetate extract of *Urtica dioica* L. induced the formation of cavities in *B. subtilis* subsp. *spizizenii* after 36 h treatment.

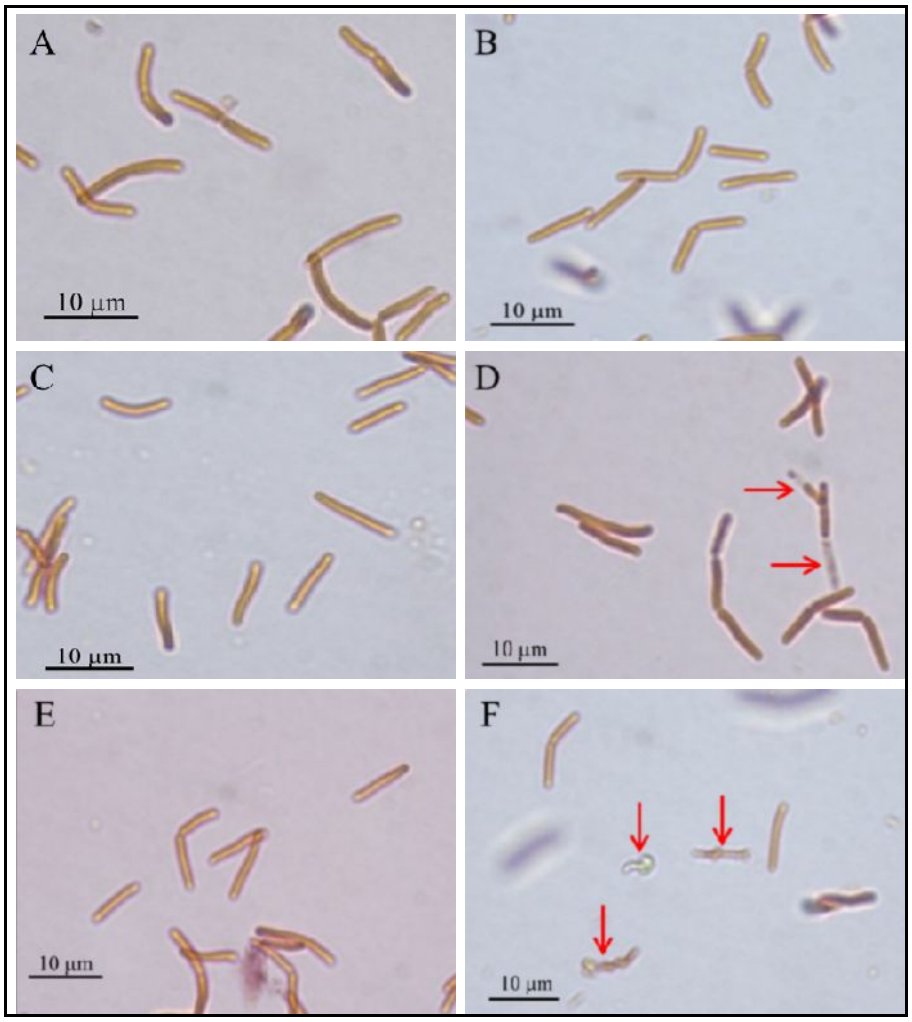

Fig. 4: *B. cereus* ATCC 11778 cell morphology after treatment with mycelial extract at 2xMBC (C and D) and 100 mg/ml (E and F) at 0 h (A, C, E) and 2 h (B, D, F) compared with the control (A and B) as observed by light microscopy at 100X. Bars = 10 µm. Arrow indicates the location of abnormal bacterial cells.

Bacterial cells treated with 2xMBC (20 mg/mL) of the culture filtrate extract for 2 h appeared elongated (Fig. 5). This finding correlates with the results of Engels *et al*.[21], who found that hepta-*O*-galloyglucose from *Mangifera indica* L. caused *B. subtilis* cells to become elongated. Khusro *et al*.[22] also found that the length of *B. subtilis* cells increased when incubated with 0.5xMIC levels of *Allium sativum* extract. This elongation may be due to incomplete cell division and/or chromosome replication. Ulanowska *et al*.[23] suggest that cell elongation is due to inhibition of DNA and RNA synthesis causing incomplete cell division.

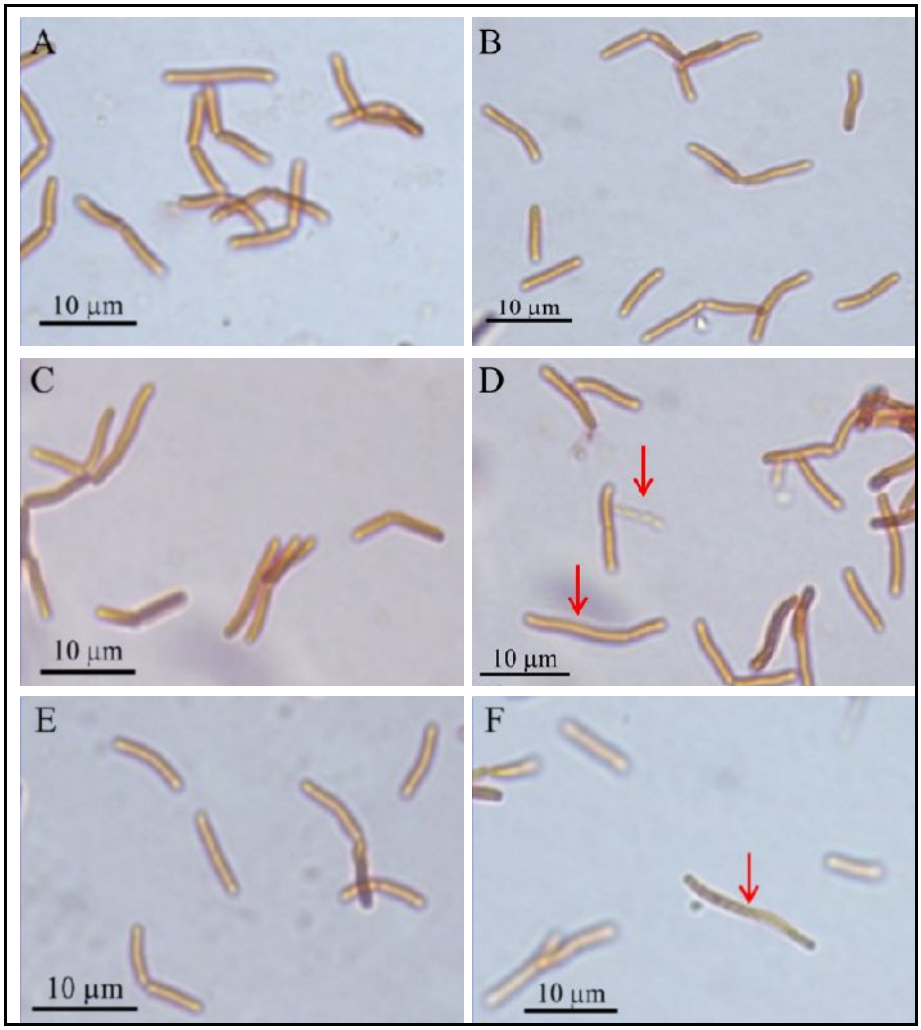

Fig. 5: *B. cereus* ATCC 11778 cell morphology after treatment with culture filtrate extract at 2xMBC (C and D) and 20 mg/ml (E and F) at 0 h (A, C, E) and 2 h (B, D, F) compared with the control (A and B) as observed by light microscopy 100X. Bars = 10 µm. Arrow indicates the location of abnormal bacterial cells.

Conclusion

Extracts derived from *Streptomyces* sp. SRF1 in the current study are antibacterial and have low MIC and MBC values. These extracts are rapidly bactericidal at 4xMBC. *Streptomyces* sp. SRF1 represents a potentially new source of antibiotics that could be developed as therapeutic agents.

Acknowledgements

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.

References

1. Bottone E.J., *Bacillus cereus*, a volatile human pathogen, Clin. Microbiol. Rev., 2010, 23, 382-398.
2. Luna V.A., King D.S., Gulledge J., Cannons A.C., Amuso P.T. and Cattani J., Susceptibility of *Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides* and *Bacillus thuringiensis* to 24 antimicrobials using Sensititre® automated microbroth dilution and Etest® agar gradient diffusion methods, J. Antimicrob. Chemoth., 2007, 60, 555–567.

3. Ikeda M., Yagihara Y., Tatsuno K., Okazaki M., Okugawa S. and Moriya K., Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections, Ann. Clin. Microbiol. Antimicrob., 2015, 14, 43.
4. Turnbull P.C., Sirianni N.M., LeBron C.I., Samaan M.N., Sutton F.N., Reyes A.E. and Peruski L.F.Jr., Mics of selected antibiotics for *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus mycoides* from range of clinical and environmental sources as determined by the Etest, J. Clin. Microbiol., 2004, 42, 3626-3634.
5. Ceylan O., Okmen G. and Ugur A., Isolation of soil *Streptomyces* as source antibiotics active against antibiotic-resistant bacteria, Eurasia. J. BioSci., 2008, 2, 73-82.
6. Yamamura H., Ashizawa H., Hamada M., Hosoyama A., Komaki H., Otoguro M., Tamura T., Hayashi Y., Nakagawa Y., Ohtsuki T., Fujita N., Ui S. and Hayakawa M., *Streptomyces hokutonensis* sp. nov., a novel actinomycete isolated from the strawberry root rhizosphere, J. Antibiot. (Tokyo), 2014, 67, 465-470.
7. Procópio R.E.L., Silva I.R., Martins M.K., Azevedo J.L. and Araújo J.M., Antibiotics produced by *Streptomyces*, Braz. J. Infect. Dis., 2012, 16, 466–471.
8. Oskay M., Antifungal and antibacterial compounds from *Streptomyces* strains, Afr. J. Biotechnol., 2009, 8, 3007-3017.
9. Yokomizo K., Miyamoto Y., Nagao K., Kumagae E., Habib E.E., Suzuki K., Harada S. and Uyeda M., Fattiviracin A1, a novel antiviral agent produced by *Streptomyces microflavus* strain No. 2445 II. Biological Properties, J. Antibiot., 1998, 51, 1035-1039.
10. Singh M.P., Petersen P.J., Weiss W.J., Janso J.E., Luckman S.W., Lenoy E.B., Bradford P.A., Testa R.T. and Greenstein M., Mannopeptimycins, new cyclic glycopeptide antibiotics produced by *Streptomyces hygroscopicus* LL-AC98: antibacterial and mechanistic activities, Antimicrob. Agents Chemother., 2003, 47, 62-69.
11. Ezra D., Castillo U.F., Strobel G.A., Hess W.M., Porter H., Jensen J.B., Condron M.A.M., Teplow D.B., Sears J., Maranta M., Hunter M., Weber B. and Yaver D., Coronamycins, peptide antibiotics produced by a verticillate *Streptomyces* sp. (MSU-2110) endophytic on *Monstera* sp., Microbiol. 2004, 150, 785–793.
12. Bae M., ChungB., Oh K., Shin J. and Oh D., Hormaomycins B and C: New antibiotic cyclic depsipeptides from a marine mudflat-derived *Streptomyces* sp., Mar. Drugs., 2015, 13, 5187-5200.
13. Sangdee A., Kornphachara S., and Srisawat N., *In vitro* screening of antagonistic activity of soil *Streptomyces* against plant pathogenic fungi and assessment of its characters, J. Agri. Tech., 2016, 12, 173-185.
14. Cwala Z., Igbinosa E.O. and Okoh A.I., Assessment of antibiotics production potentials in four actinomycetes isolated from aquatic environments of the Eastern Cape Province of South Africa, Afr. J. Pharm. Pharmacol., 2011, 5, 118-124.
15. Sharmin T., Rahman M.A., Anisuzzaman A.S.M. and Islam M.A., Antimicrobial and cytotoxic activities of secondary metabolites obtained from a novel species of *Streptomyces*, Bangladesh Pharm. J., 2013, 16, 15-19.
16. Taechowisan T., Chanaphat S., Ruensamran W. and Phutdhawong W.S., Antibacterial activity of decursin from *Streptomyces* sp. GMT-8; an endophyte in *Zingiber officinale* Rosc, J. Appl. Pharm. Sci., 2013, 3, 74-78.
17. Sim J.H., Jamaludin N.S., Khoo C.H., Cheah Y.K., Halim S.N.B.A., Seng H.L. and Tiekink E.R.T., *In vitro* antibacterial and time-kill evaluation of phosphanegold (I) dithiocarbamates, $R_3PAu[S_2CN(iPr)CH_2CH_2OH]$ for R = Ph, Cy and Et, against a broad range of Gram-positive and Gram-negative bacteria, Gold Bul., 2014, 47, 225-236.
18. Ultee A., Gorris L.G.M. and Smid E.J., Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*, J. Appl. Microbiol., 1998, 85, 211-218.
19. Messick C.R., Rodvold K.A. and Pendland S.L., Modified time-kill assay against multidrug-resistant *Enterococcus faecium* with novel antimicrobial combinations, J. Antimicrol. Chemoth., 1999, 44, 831-834.
20. Chahardehi A.M., Ibrahim D., Sulaiman S.F. and Mousavi L., Time-kill study of ethyl acetate extract of stinging nettle on *Bacillus subtilis* subsp. *spizizenii* ATCC CRM-6633 strain NRS 231, Annu. Res. Rev. Biol., 2015, 6, 33-40.

21. Engels C., Schieber A. and Gänzle M.G., Inhibitory spectra and modes of antimicrobial action of gallotannins from mango kernels (*Mangifera indica* L.), Appl. Environ. Microbiol., 2011, 77, 2215-2223.
22. Khusro A., Preetam R.J.P. and Panicker S.G., Adaptational changes in cellular morphology of *Bacillus subtilis* strain KPA in response to certain antimicrobials, Int. J. ChemTech Res., 2014, 6, 2815-2823.
23. Ulanowska K., Tkaczyk A., Konopa G. and Węgrzyn G., Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains, Arch. Microbiol., 2006, 184, 271–278.

*****