Screening and partial purification of antibacterial compounds from *Streptomyces* sp. strain RS2†

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ABSTRACT

Streptomyces sp. RS2 was isolated from soil samples in Dong Pong village, KhonKaen province, Thailand. The active strain RS2 was identified as genus *Streptomyces*, and it was related to *S. gelaticus* NRRL B-2928T at 99.43% 16S rDNA similarity. It was screened for antifungal and antibacterial activities by dual culture and cross streak methods, respectively. The results showed that *Streptomyces* sp. RS2 exhibited anti-microbial activity against fungal and gram-positive and gram-negative bacteria. Therefore, it was cultured on ISP2 medium at 30°C for 7 days. After incubation, the culture medium was dried, and the mashed biomass was extracted with organic solvents as methanol (MeOH). The crude MeOH extract was purified by using a high performance liquid chromatography (HPLC) with diode-array detector (DAD). Additionally, the partial purified fractions were tested for antibacterial activity by agar well diffusion method. The results showed that 5 fractions showed antibacterial activity against S. aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia* coli ATCC 25922. The fraction number 3 and 4 exhibited the highest antibacterial activity, these fractions will be interested to purify and study in the function of the active compounds in the future.

Keywords: *Actinomycetes*, *Streptomyces* sp. RS2, Antimicrobial activity, S. aureu

1. INTRODUCTION

Actinomycetes are filamentous gram positive bacteria. They have a high guanine and cytosine (G+C) content in their DNA [1]. Generally, they are free living and commonly distributed in aquatic and terrestrial environment [2]. *Actinomycetes*, especially *Streptomyces*, have variety of applications in biodegradation, industrial, biotransformation, environmental, agri-cultural, and pharmaceutical fields [3]. Approximately 70% marketed antibiotics are derived from the active compounds produced by genus *Streptomyces* such as S. Aureofaciens which is an important producer of chlortetracycline and tetracycline [4]. The majority interest in this genus is the ability to produce many important bioactive [5]. They have produced a wide range of clinically important antibiotics, including strepto-mycin, tetracycline, neomycin and erythromycin [6]. These compounds are used not only in the treatment of various

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human and animal diseases but also in agriculture and biochemistry. Currently, gram-positive and gram-negative pathogens are the most important causes of infections in hospitals such as surgical site, bloodstream, urinary tract, pneumonia and skin infections. However, microbial natural products have been one of the most important sources of novel bioactive compounds. Many researches have focused on the broad-spectrum antibacterial and antifungal activity of the crude extracts and purified compounds from the genus *Streptomyces*. Additionally, this genus continues to play major role in the novel bioactive compounds production. Therefore, the objectives of this study were to screen, extract and partial purify antimicrobial agents of *Streptomyces* sp. isolated from soil samples.

2. MATERIALS AND METHODS

2.1 *Streptomyces* sp. RS2

Streptomyces sp. RS2 was isolated from soil sample, and it was classified as genus *Streptomyces* based on phenotypic, genotypic and phylogenetic analysis [7]. The strain RS2 was identified based on phenotypic characters such as morphological, cultural and biochemical characteristics was studied by following the methods of Mangamuri et al. [8]. PCR amplification of 16S rDNA gene was performed using 20F (5'-GAGTTTGATCCTGGCTCAG-3') and 1500R (5'-GTTACCTTGTTACGACTT-3') as forward and reverse primers, respectively. Amplified DNA was purified and submitted to sequencing by National center for genetic engineering and biotechnology (BIOTEC).16S rDNA sequence was analyzed using the BLASTN search tools and EzTaxon-e server. The phylogenetic tree was constructed by the neighbour-joining method by using the software package MEGA version 6.

2.2 Production and extraction of anti-microbial agent

Streptomyces sp. RS2 was cultured on ISP2 medium and incubated at 30°C for 7 days. After incubation, the culture medium was dried and the mashed biomass was extracted with methanol (3x1L, MeOH) [9]. Crude MeOH extract was evaporated to dryness by speed-vac™ concentrator (LaboGene) at Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. The crude extract was stored at -20°C.

2.3 Partial purification of the crude MeOH extract by HPLC-DAD analysis

The crude extract was partial purified by using a high-performance liquid chromatography with diode-array detector (HPLC-DAD). The solution of sample (1 µl) was injected into the HPLC column (10 X 250 mm, filled with Nucleosil-100 C-18 (5 m). Separation was performed by a linear gradient using 0.1% ortho-phosphoric acid as solvent A and acetonitrile as solvent B. The gradient was used from 0 to 100% solvent B in 15 min at a flow rate of 2 ml/min. Multiple wavelength monitoring were performed at 210, 230, 260, 280, 310, 360, 435 and 500 nm, without reference wavelength. The UV visible spectrum was measured from 200 to 600 nm. The partial purified fractions were collected and screened for antimicrobial activity against reference bacteria as S. aureus ATCC 25923, P. aeruginosa ATCC 27853 and E. coli ATCC 25922 by agar well diffusion method [10].

2.4 Testing for antibacterial activity by agar well diffusion method

The partial purified fractions were tested for antibacterial activity by agar well diffusion method on Mueller hinton agar (MHA) [11, 12]. The reference bacteria as S. aureus ATCC 25923, P. aeruginosa ATCC 27853 and E. coli ATCC 25922 were inoculated into Mueller hinton broth (MHB) and incubated at 37°C, for 4-

6 h. The culture broth were adjusted the turbidity to equal the 0.5 McFarland standard, and then three dimensions swab were prepared on a MHA medium. After that, wells were prepared and cut out by a sterile cork-borer. The crude extracts and partial purified fractions (30 µg/well) were dissolved in 25% MeOH at final concentration. These solutions (20 µL) were loaded into each well and incubated at 37°C for 24 h [13]. The diameter of inhibition zone (mm) was reported after three repeats.

2.5 Scanning electron microscopy (SEM)

S. aureus ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 cells in exponential phase were treated with the active partial purified fractions (100 µg/well), incubated at 37°C for 2-8 h. Cells without the active fractions were used as controls. Bacterial cells were collected by centrifugation at 4°C, 5000 for 10 min, and washed gently with phosphate buffer saline (PBS, 0.1 M, pH 7.4). Subsequently, the tested cells were fixed in 2.5% glutaraldehyde solutions at 4°C for 2-3 h and dehydrated with gradient ethanol solutions (30, 50, 60, 70, 90 and two times with 100%). The cells were critical-point dried, mounted on stubs, sputter-coated with gold (gold-palladium, or platinum) and finally imaged using SEM (LEO 1450vp) at the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand.

3. RESULTS AND DISCUSSION

3 .1 Preliminary screening for anti-microbial activity

Streptomyces sp. RS2, an anti-microbial-producing bacterium, showed antifungal and antibacterial activity against 10 plant pathogenic fungi, 5 gram-positive and 4 gram-negative bacteria, respectively. Interestingly, *Streptomyces* sp. RS2 exhibited antibacterial activity against 18 clinical strains of *Staphylococcus* aureus and Methicillin-resistant *S. aureus* (MRSA). Therefore, this active isolate was selected for the further study [7]. The similar results were reported by Nguyen and Kim [14] that the *Streptomyces olivicoloratus* showed antimicrobial activities against *Bacillus subtilis, S. aureus, P. aeruginosa, S. epidermidis*, *Paenibacillus larvae, E. coli, Candida albicans and Aspergillus nige*r. Tamreihao et al. [15] reported that the *Streptomyces* sp. MBRL 10 exhibited antifungal activity against the tested fungal pathogens. The active strain MBRL 10 showed the highest activity against *Rhizoctonia solani*. Additionally, Euch et al. [16] reported that two bioactive compounds, namely 3-phenylpyrazin-2 (1H) -one (**1**) and 3-O-methylviridicatin (**2**), showed anti-bacterial activity against the tested human pathogenic bacteria as *S. aureus, L. monocytogenes* and *S. typhimurium.*

3.2 Identification of *Streptomyces* sp. RS2

The result of cultural characteristics illustrated range and optimum temperature and pH for growth were 25-40°C and 30°C; and 5-10 and 7, respectively. The range of NaCl concentration and optimum NaCl for growth were 1-8% and 1%, respectively. Based on 16S rDNA sequence and phylogenetic tree characterization, the isolate RS2 belongs to the genus *Streptomyces*. Isolate RS2 (1418 bp) showed 16S rDNA sequence similarity with *S. gelaticus* strain NRRL B-2928T (1483 bp, 99.51%), S. *sanglieri* strain NBRC 100784 (1481 bp.99.44%) and *S. atratus* strain NRRL B-16927 (1487 bp, 99.37%). The phylogenetic tree determined by neighbor-joining method was presented in Figure 1. Based on the results of cultural, physiological, and biochemical characteri- zation, the selected isolate was identified as genus *Streptomyces* and designated as *Streptomyces* sp. RS2.

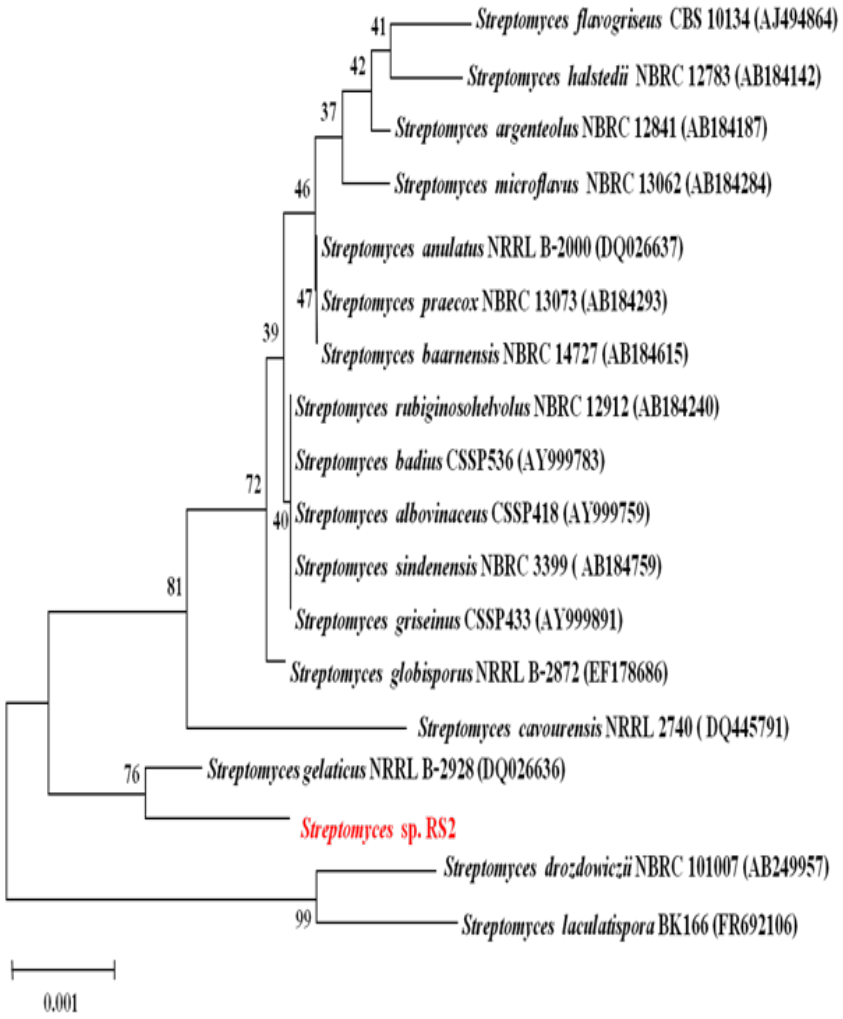

Figure. 1. Phylogenetic tree of the selected isolate RS2 based on 16S rDNA sequences using Neighbor-joining method. The numbers at the nodes indicate bootstrap support (%) based on NJ analysis of 1000 replicates. The scale bar indicates 0.001 substitutions per site (C).

3.3 Partial purification of the crude MeOH extracts and antibacterial activity

The HPLC-DAD chromatogram of the crude MeOH extract is shown in Figure. 2. All of the partial purified fractions were screened for antibacterial activity by agar well diffusion method. The results showed that the fraction number 1 (11.448 min), 2 (13.341 min), 3 (15.042 min), 4 (26.173 min) and 5 (26.462 min) exhibited antibacterial activity against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 (Figure 3 and Table 1). As the fraction number 3 and 4 exhibited the highest antibacterial activity especially against *S. aureus* ATCC 25923 and many isolates of MRSA. Moreover, *S. aureus* particularly MRSA are one of the most important human pathogen in hospital. Hence, both of these fractions will be interested to purify for further study. The similar result was reported by Rajan and

Kannabiran [10] reported that the crude ethyl acetate extract of *Streptomyces* sp. VITBRK2 was purified by HPLC-DAD analysis. The purified compounds were identified as N-Acetyl-phenylalanine, 3-methyl-indole and amicoumacin antibiotic. These active compounds exhibited antibacterial activity against drug resistant Methicillin-resistant *S. aureus* (MRSA) and vancomycin resistant Enterococci (VRE).

3.4 Effect of the active purified fractions on the tested bacterial cells

The result showed that the tested bacterial cells were slightly changed and decreased after treatment with the active purified fractions as shown in Figure. 4. The results illustrated that the active purified fractions exhibited a broad spectrum of antibacterial activity against gram positive bacteria (*S. aureus* ATCC 25923) and gram negative bacteria (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922). The cell morphology of the tested bacteria without being incubated with the active fractions showed a complete and smooth surface; while after incubating with the active fractions, the cell morphology and the cell-shapes of the tested bacteria were damaged and completely destroyed. Therefore, these results suggested that the active fractions from the crude MeOH extract of *Streptomyces* sp. RS2 may act on the bacterial cells, resulting in the inhibition of the bacterial growth. Previously reported, He et al. [17] showed that a novel polysaccharide of *S. virginia* H03 exhibited broad spectrum antibacterial activities *against S. aureus, B. subtilis, Listeria mono- cytogenes, E. coli, Zygosaccharomyces bailii* and *C. utilis. Based* on SEM observation, the polysaccharide could be destroy and disrupt the tested cell.

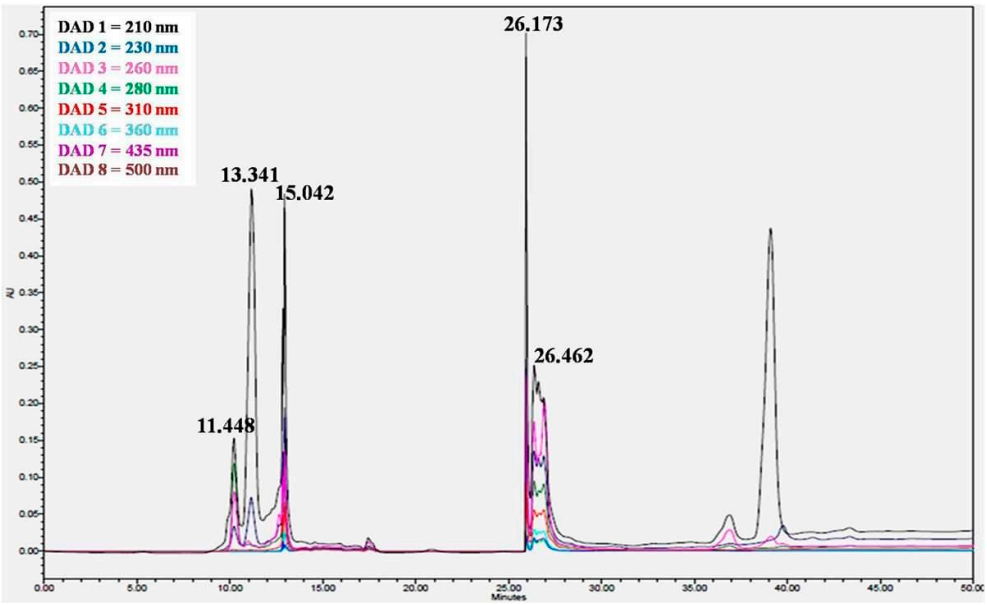

Figure. 2. The HPLC-DAD chromatogram of the crude MeOH extract from *Streptomyces* sp. strain RS2.

Table 1. Antibacterial activity of the partial purified fractions against S. aureus ATCC 25923, P. aeruginosa ATCC 27853 and E. coli ATCC 25922 on MHA medium by agar well diffusion method.

Control/ Partial purified fractions	Inhibition zone (mm)		
	***S. aureus* ATCC 25923**	***P. aeruginosa* ATCC 27853**	***E. coli* ATCC 25922**
C	NI	NI	NI
Frac. 1	11.21± 0.04	3.15± 0.35	NI
Frac. 2	12.02± 0.15	4.11± 0.21	2.05± 0.55
Frac. 3	22.51± 0.74	9.65± 0.55	5.14± 0.63
Frac. 4	21.02± 0.05	8.51± 0.34	4.35± 0.57
Frac. 5	15.53± 0.32	5.65± 0.25	3.01± 0.59

Values are means of three replicates ± standard deviation (SD), NI; no inhibition; C, control (25% MeOH); Frac. 1, fraction 1; Frac. 2, fraction 2; Frac. 3, fraction 3; Frac. 4, fraction 4 and Frac. 5, fraction 5

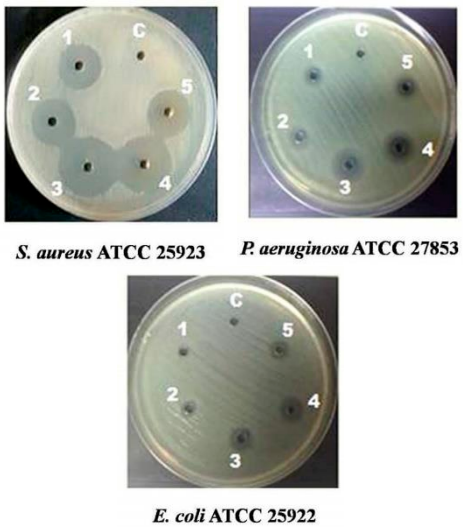

Fig. 3. Antibacterial activity of the active fractions from isolate RS2 against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922

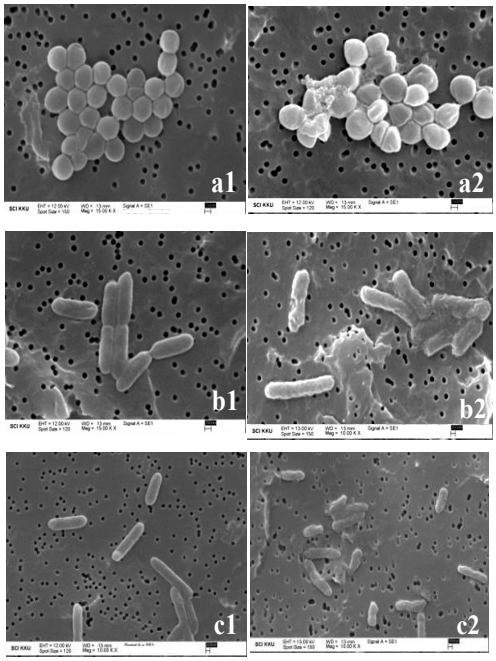

Fig. 4. SEM micrograph of pathogenic bacteria after treated with the active purified fractions. a1-a3: *S. aureus* ATCC 25923, b1-b3: *E. coli* ATCC 25922 and c1-c3: *P. aeruginosa* ATCC 27853, 1 bacterial cells without treating the active fraction; 2 treating with the active fractions

4. CONCLUSIONS

Streptomyces sp. RS2 is an antimicrobial-producing strain that exhibited the highest antifungal and antibacterial activity. Interestingly, five partial purified fractions exhibited antibacterial activity against *S. aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. The results of this study suggest that the active *Streptomyces* sp. RS2 is a potential strain capable of producing bioactive compounds and could be used these active compounds against drug and multi-drug resistant bacteria.

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