농학석사학위논문

방선균 *Streptomyces bacillaris* MBTH40 대사산물의 항균활성 연구

Antimicrobial Metabolites from *Streptomyces bacillaris* MBTH40

2020년 2월

서울대학교 대학원

농생명공학부 응용생명화학전공

강새연

A Dissertation for the Degree of Master of Science

Antimicrobial Metabolites from *Streptomyces bacillaris* MBTH40

February 2020

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이 논문을 농학석사학위논문으로 제출함

2019년 12월

서울대학교 대학원

농생명공학부 응용생명화학전공

강 새 연

강새연의 석사학위논문을 인준함

2020년 1월

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Abstract

Actinobacteria is an attractive phylum to produce diverse secondary metabolites with high utility as a source of anti-infectives. Marine-derived actinobacteria is relatively unrevealed realm due to the low accessibility. Since the factors including pressure, light, temperature and concentration of oxygen are extremely various compared to the soil environment, marine actinobacteria are expected to produce metabolites with novel structures and mode of actions. The sediment of the deep sea located in the Pacific Ocean was provided by Korea Institute of Ocean Science & Technology (KIOST). In this study, 406 actinobacteria isolates from the sediment were screened and *Streptomyces bacillaris* MBTH40 was selected guided by the inhibitory activity against *Staphylococcus aureus.* Through subsequent extraction and separation, four compounds were isolated, and the structures were elucidated via spectroscopic analyses. All the compounds were revealed as known materials. Afterwards, antibacterial, antifungal and isocitrate lyase (ICL) inhibition activities of each compound were investigated. The results present notable inhibitory activity of lactoquinomycin A against pathogenic bacteria including MRSA strains. None of the compounds exhibited antifungal activity, while lactoquinomycin B was found to moderately inhibit activity of ICL.

Key words: Marine-derived actinobacteria, secondary metabolites, *Streptomyces bacillaris*, antimicrobial activity, MRSA

Student Number: **2018-27278**

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List of Abbreviations

ACN	Acetonitrile
AMP	Ampicillin
aq.	Aqueous
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
CAS	Chemical Abstracts Service
CCARM	Culture Collection of Antibiotic Resistant Microbes
CFU	Colony forming unit
CIP	Ciprofloxacin
CLSI	Clinical & Laboratory Standards Institutes
COSY	Correlation spectroscopy
gDNA	Genomic deoxyribonucleic acid
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single quantum coherence spectroscopy
IC_{50}	The half maximal inhibitory concentration
IDSA	Infectious Diseases Society of America
IFM	Research Center for Pathogenic Fungi and Microbial Toxicoses
KIOST	Korea Institute of Ocean Science & Technology

LB	Luria-Bertani broth
LC-MS	Liquid chromatography-mass spectrometry
LQM-A	Lactoquinomycin A
LQM-B	Lactoquinomycin B
MeOH	Methanol
MHB	Muller Hinton broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
m/z	Mass-to-charge ratio
NADH	reductive state of nicotinamide adenine dinucleotide
NBRC	Biological Resource Center, National Institute of Technology and Evaluation (NITE)
NCBI	National Center for Biotechnology Information
NICEM	National Instrumentation Center for Environmental Management
NMR	Nuclear magnetic resonance
OD	Optical density
PCR	Polymerase chain reaction
PBS	Phosphate-buffered saline
RI	Refractive index
RPMI	Roswell Park Memorial Institute
rRNA	Ribosomal ribonucleic acid
TET	Tetracycline
TFA	Trifluoroacetic acid

TMS	Tetramethylsilane
t_R	Retention time
Tris	Tris(hydroxymethyl)aminomethane
VAN	Vancomycin
WHO	World Health Organization
YPD	Yeast extract peptone dextrose

Introduction

Antibiotic refers to a molecule which inhibits or kills bacteria. After the introduction of penicillin in early 1940s, the “antibiotic era” began, saving millions of lives against infectious diseases (Aminov, 2010; Lobanovska, 2017). Still, antibiotic is regarded as the most successful chemotherapeutic agent in modern medicine. Human’s mortality rate associated with bacterial infections was decreased notably and the advances in medical operations including organ transplantation have become possible as well (Gould and Bal, 2013). Approximately half of the antibiotics used these days were discovered between 1950 and 1960, undoubtedly the golden era of antibiotics (Davies, 2006). Mostly, natural products were the major source of chemotherapeutic agents including antibiotics. Comparing the percentages turned to drugs, natural products were far more efficient than that of synthetic chemicals (Berdy, 2012). Specifically, the compounds from microorganism accounted for the largest proportion. Structural complexity and diversity of natural products have been provided highly useful scaffolds for not only anti-infection molecules, but also antitumor, antiparasitic molecules (Berdy, 2012).

Actinobacteria is a phylum of gram-positive bacteria. Beside their role as a member of the eco system, actinobacteria has an importance due to their ability to produce various secondary metabolites possessing useful bioactivities including antibacterial, antifungal, anticancer activities. For instance, while 22 new antibiotics were launched after 2000, 12 of them were natural derived, mostly from

actinomycetes (Butler et al., 2013). Although soil-dwelling actinobacteria have been researched vigorously, marine-derived actinobacteria has been relatively underexploited due to the low accessibility. Since the marine actinobacteria have been adapted to the environments with wide range of temperature, pressure, salinity, light and concentration of oxygen, they are expected to produce abundant novel molecules with unique structure and mechanism. Among the actinobacteria, exclusively *Streptomyces* genus produced approximately 80% of antibiotics such as streptomycin from *Streptomyces griseus,* tetracycline from *Streptomyces aureofaciens,* vancomycin from *Streptomyces orientalis,* etc., attracting significant interests (Procopio et al., 2012).

The number of resistant bacteria strains is increasing fast and it is becoming more serious threat to global health. CDC (Centers for Disease Control and Prevention) described the current situation, exhausted pipeline and resistant crisis, as "post-antibiotic era" in 2013. Recently, over 70% of pathogenic bacteria are resistant to majority of antibiotics available on market (Berdy, 2012). Globally, at least 70,000 deaths are caused by resistant infections annually, and it will get worsened without alternatives (Crofts et al., 2017). Abuse and misuse of antibiotics in clinical and agricultural conditions have attributed to the arise and spread of resistance (Alanis, 2005). Microbes have acquired an incredible adaptability and flexible metabolic power which enable them to develop resistance to any kind of antibiotics (Berdy, 2012). Unlike other agents prescribed for various diseases (e.g. diabetes and high blood pressure), antibiotics have relatively short valid period about five to seven decades or less (Gould and Bal, 2013). As an example, resistance against

sulfonamides, the first widely-used antibiotics, was occurred only six years after the mass introduction (Crofts et al., 2017). On the contrary, decelerated rate of drug discovery increase the severity. Since 1960s, none of new class of antibiotics with wide-spectrum of derivatives has been documented (Lewis, 2013). Then the time after late 1980s, it is called discovery voids (WHO, 2014).

Staphylococcus aureus is one of the 'ESKAPE' pathogens (*Enterococcus* spp., *Staphylococcus aureus, Klebsiella* spp., *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) which has high frequency of resistance and prevalence identified by the IDSA (Boucher et al., 2009). *S. aureus* is an opportunistic pathogen which is a leading cause of bacteremia, endocarditis, osteomyelitis and skin and soft-tissue infections (SSTIs) (Turner et al., 2019). As Fleming's awareness of the resistance, *S. aureus* soon developed the resistance against penicillin and other β-lactams by encoding β-lactamase or PBP with lower affinity to the drug (Olsen et al., 2006). Since MRSA was first emerged in 1961, the strain resistant to vancomycin, which was the last available treatment, was reported on 2002 (Jevons, 1961; Chang et al., 2003). Acquisition of resistance is danger not only because it requires complicated treatment, but also it is associated with enhanced virulence (Davies, 2006). Furthermore, most of genes contributing to virulence or resistance are encoded on insertion elements (ISs), transposons, plasmids, prophages, and genomic islands which have high probability to be transferred among both intraspecies and interspecies (Gill et al., 2005; Kennedy et al., 2008).

Antibiotics and other inhibitors have its own targets and mechanisms. To achieve

a successful therapeutic intervention, these molecules should target essential components of the pathogen which are absent in host cells. For instance, β-lactams inhibit bacterial cell wall transpeptidases and tetracyclines target bacterial ribosomes, both do not exist in human cells (Chopra and Roberts, 2001; Konaklieva, 2014). Alternatively, anti-virulence molecules can be used to combat pathogens without killing bacteria. Virulence is an ability of pathogens to harm host by colonizing, infecting and persisting in susceptible host (Fleitas Martinez et al., 2019). Isocitrate lyase is an attractive target of anti-virulence drugs since it is an essential enzyme for the pathogens to be fully virulent. Isocitrate lyase (ICL) is a key enzyme consists glyoxylate cycle, catalyzing the cleavage of isocitrate into succinate and glyoxylate. In a following step, glyoxylate is assimilated into malate by malate synthase (MS), incorporating C_2 carbon sources to tricarboxylic acid cycle. It is well documented that ICL plays important roles in virulence of *Candida* genera, *Mycobacterium tuberculosis*, and so on (McKinney et al., 2000; Ramirez and Lorenz, 2007). On the other hand, strategies using adjuvants, QS-targeting molecules, probiotics and various approaches are being studied (Spellberg and Gilbert, 2014; Theuretzbacher et al., 2019). Since these methods exert lower selective pressure to microbes than that of traditional antibiotics, the frequency of resistance is lower as well.

In this regard, integrated approaches encompassing development of antimicrobials and stewardship practices should be accounted to combat pan-drug-resistant organisms (Crofts et al., 2017; Theuretzbacher and Piddock, 2019). This study is directed toward discovering bioactive molecules from *Streptomyces*. Natural products have the advantages of structural diversity, steric complexity, multiple

chiral centers and high efficiencies with multiple modes of action (Berdy, 2012). It is important to rekindle novel antimicrobial discovery to provide various scaffolds which can be applied in various aspects. And as aforementioned, the natural products can be the most promising source.

The purpose of this study is to extract bioactive compound from marine-derived actinobacteria. Actinobacteria species from the sediment of the Pacific Ocean were provided by KIOST.

Materials and Methods

Screening of marine-derived actinobacteria

For screening of 406 species of marine-derived actinobacteria provided from KIOST, top agar overlay assay was conducted. They were temporarily named as 1A to 41J. Each species was streaked in slant seed50 media (5 g of glucose, 10 g of starch, 5g of peptone, 2 g of yeast extract, and 17 g of sea salt (half of the sea salt concentration) per 1 L distilled water). After incubation for two weeks in 28℃, tween 80 10% solution was added to harvest the spores. On the CM50 agar (5 g of yeast extract, 5 g of bacteriological peptone, 10 g of glucose, 10 g of soluble starch, 17 g of sea salt, and 20 g of agar per 1 L distilled water), GTYB50 agar (10 g of glucose, 2 g of tryptone, 1 g of yeast extract, 1 g of beef extract, 17 g of sea salt, and 20 g of agar per 1 L distilled water), YPM50 agar (2 g of yeast extract, 2 g of bacteriological peptone, 4 g of mannitol, 17g of sea salt, and 20g of agar per 1 L distilled water), 1 μL of the aliquot was dotted respectively. After incubation for two days at 28℃, top agar (0.35%, MHB) containing 5×10^5 CFU/mL of *S. aureus* ATCC 25923 was inoculated. To determine inhibitory activity against *S. aureus,* the inhibition zone was observed in naked eyes after 16-20 h incubation at 37℃. Reproducibility, and efficacy of inhibitory activity were considered to select both bacteria and media for further cultivation.

Identification of *Streptomyces bacillaris* MBTH40

To identify the strain 40H (identified later as *S. bacillaris* MBTH40), 16S rRNA gene sequence was analyzed. To extract gDNA from mycelium of YPM50 grown 40H, i-Genomic BYF DNA Extraction Mini Kit (Intron, Republic of Korea) was used. Subsequently, 16S rDNA was amplified via PCR. Reaction mixture of PCR includes 10 μL of 5×PrimeSTAR buffer (Mg^{2+} plus), 4 μL of dNTP mixture (2.5 mM each), template DNA (< 200 ng), 27F and 1525R primers (final concentration 0.2 – 0.3 μM each), and 0.5 μL of PrimeSTAR HS DNA polymerase (2.5 U/μL) filled with distilled water to reach 50 μL. PCR starts with 1 min initial denaturation at 98℃ followed by 30 cycles consist of 10 sec at 98℃ for denaturation, 5 sec at 55℃ for annealing, and 1.4 min at 72℃ for extension. Amplified sequence was aligned through NCBI BLAST. MEGA X software was used to draw neighbor joining tree based on the sequence data from NCBI. The primers used to obtain 16S rRNA gene segment are as follows: 27F, 5-AGAGTTTGATCCTGGCTCAG-3; and 1525R, 5-AAGGAGGTGATCCAGCC-3.

Cultivation of *S. bacillaris* MBTH40

Glycerol stock (15% glycerol) containing spore aliquot of *S. bacillaris* MBTH40 was preserved in deep freezer at -80℃. The aliquot of MBTH40 stock was streaked on seed50 agar plate and incubated for two weeks at 28℃ to attain the spores. Then the spores were inoculated into 25 mL of YPM50 media in 200 mL Erlenmeyer flask for two days. Each seed broth was transferred to 500 mL of YPM50 media in 2 L

Erlenmeyer flask and fermented for five days. All the liquid cultivations were processed in shaking incubator with rpm 120 at 25℃ and every medium was autoclaved for 15 min at 121℃ before the cultivation.

Extraction and separation of metabolites from strain MBTH40 culture

Fermented supernatant was filtered by filter paper (300 mm, Hyundai Micro, Seoul, Korea). In liquid-liquid extraction, filtrates were partitioned by same volume of ethyl acetate for two times. Organic layer was collected, and the solvents were removed under reduced pressure. The residue was dissolved in distilled water and repartitioned with *n*-hexane to remove hydrophobic compounds. Finally, dichloromethane extract was obtained. After the solvent removal, the crude extracts were dissolved in HPLC grade MeOH to be subjected to semi-preparative reverse-phase HPLC. RP-HPLC was equipped with TRILUTION LC control software with a 321 pump, UV/VIS-151 detector (Gilson, Middleton, WI, USA), and Agilent Eclipse XDB C_{18}, 5 μm, (9.4 × 250 mm) with guard column. The elution was monitored by the UV absorption at 254 nm and the flow rate was 2 mL/min. Mobile phase setting for the crude extract was 10% to 100% (v/v) aq. MeOH (H_2O with 0.1% TFA added) and the running time was 30 min. All the solvents used in HPLC were HPLC grade purchased from Thermo Fisher Scientific. Each fraction from HPLC was purified in other suitable mobile conditions.

Purification of the compounds

The fractions from the semi-preparative HPLC were successively purified under different mobile settings. HPLC apparatus were same as mentioned previously. Isocratic condition was used to purify P1, P2 and P4-3. The solvent system was 20% aq. ACN including 0.1% TFA for P1 and P4-3, and 30% aq. ACN with 0.1% TFA for P2, respectively. Only P5 was purified under gradient mobile setting consists of 10% to 100% aq. ACN (H_2O containing 0.1% of TFA) in 30 min.

Structure elucidation

The structures of the compounds from MBTH40 were elucidated via ^{1}H, ^{13}C and 2D NMR (COSY, HMBC, and HSQC) analysis data measured by Bruker AVANCE 600 spectrometer (Bruker BioSpin Ltd., Germany) located in NICEM, Seoul national university, Seoul, Korea. Purified samples were dissolved in 600 μL of methanol-d_4 or the mixture of methanol-d_4 and dimethyl sulfoxide-d_6. Dissolved samples were contained in 5 mm NMR tube (Wilmad-LabGlass, NJ, USA) and then subjected to analysis.

Electrospray ionization (ESI) spectra in both positive and negative ion modes were measured. Mass spectra and UV spectra were provided by the Laboratory of Natural Products and Structure Determination, Seoul National University, Korea.

Antibacterial activity assay

General procedures were guided by CLSI method (CLSI, 2018). On 96 well plate containing MHB, samples were added to the first column followed by a two-fold serial dilution. Concentration of samples ranged from 128 μg/mL to 0.015 μg/mL. Growth control, sterile control, negative and positive controls were included in each well plate to validate the results. Target bacteria were pre-incubated in MHB agar for a day and colonies were incubated in 5 mL MHB to be subjected to assays. When inoculating bacteria, McFarland 0.5 was measured using spectrometry at UV 625 nm wavelength. The final concentration of the inoculated bacteria was 5×10^5 CFU/mL. After 16-20 h incubation, MIC was determined as the lowest concentration with no visible bacterial cell growth. *S. aureus* ATCC 25923, *Enterococcus faecium* ATCC 19434, *Enterococcus faecalis* ATCC 19433, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, and *Salmonella enterica* ATCC 14028 were used as test strains. Ampicillin and tetracycline were used as positive controls. As MRSA strains, *S. aureus* ATCC 43300, *S. aureus* ATCC 700787, *S. aureus* ATCC 700788, *S. aureus* CCARM 3089, *S. aureus* CCARM 3634, *S. aureus* CCARM 3090, *S. aureus* CCARM 33591, and *S. aureus* CCARM 3635 were subjected to anti-MRSA assay. Ciprofloxacin and vancomycin were used as positive controls for these MRSA strains.

Antifungal activity assay

The protocol was instructed by CLSI protocol (CLSI, 2017). On 96 well plate

containing RPMI 1640 (Sigma) whose pH was adjusted to 7, samples were added to the first column followed by a two-fold serial dilution as well. Concentration of samples ranged from 128 μg/mL to 0.25 μg/mL. *Candida albicans* ATCC 10231, *Aspergillus fumigatus* HIC 6094, *Trichophyton rubrum* NBRC 9185, and *Trichophyton mentagrophytes* IFM 40996 were grown in PDA (Acumedium Manufacturers, Inc., Maryland) agar for 3-4 days before they are subjected to the assay. *C. albicans* were pre-incubated in YPD agar (10 g of yeast extract, 20 g of peptone, 20 g of glucose, and 20 g of agar per 1 L distilled water) for a day and colonies were diluted in PBS for cell count. *A. fumigatus, T. rubrum,* and *T. mentagrophytes* were incubated for 3 - 4 days to attain spores. Approximate CFUs of *C. albicans* and *A. fumigatus* were measured based on McFarland parameter 0.5 at UV 530 nm wavelength. Then the cells were diluted to RPMI 1640 to reach the final concentration 0.4 – 5×10^4 CFU/mL. For *T. rubrum* and *T. mentagrophytes*, the number of spores were counted using hemocytometer and microscope. Spores were also diluted in RPMI 1640 to reach the final concentration 0.5 – 2.5×10^3 CFU/mL. Amphotericin B was used as a positive control. MICs were determined as a concentration with no visible fungal cell growth as well.

ICL inhibition assay

Pre-incubated transformant *E. coli* were inoculated in 400mL LB medium containing ampicillin (50 μg/mL) and incubated at 37°C for 16 h. When OD_{600} was measured as 0.5, L-arabinose (final concentration: over 0.02%) was added for induction followed by additional incubation at 25°C for 8 h. Ni-NTA affinity column chromatography

(Qiagen, Hilden, Germany) was conducted to purify His-tagged ICL.

To examine enzyme inhibition activity, assay was conducted according to the previously documented procedures (Dixon and Kornberg, 1959). Composition of the enzyme reaction buffer is 20 mM sodium phosphate buffer (pH 7.0), 1.27 mM threo-DL (+) isocitrate, 3.75 mM $MgCl_2$, 4.1 mM phenylhydrazine and 2.5 μg/mL purified ICL. Test compounds were serially diluted followed by 30 min incubation in 37℃. To measure the activity of ICL, formation of glyoxylate phenylhydrazone was detected at 324 nm wavelength by spectrometer. Based on the inhibition ratio, IC_{50} of each compound was calculated by non-linear regression analysis (Graph Pad). Known ICL inhibitor, 3-nitropropionate, was used as a reference compound.

Results

Selection and cultivation of 40H

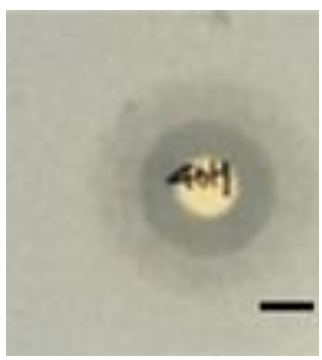
Screening of 406 species revealed that 24 species exhibited mild or strong inhibition against *S. aureus.* Guided by the anti-staphylococcal activity, 40H was selected for this study. According to the result of top agar overlay assay, the size of the inhibition zones from GTYB50 and YPM50 were similar (Fig. 1). Therefore, MICs of culture extracts from both GTYB50 and YPM50 were examined to figure out the most suitable media for 40H to produce anti-staphylococcal metabolites (Table 1). YPM50 culture extract exhibited MIC of 8 μg/mL lower than 16 μg/mL of GTYB50 culture extract. According to the result, YPM50 was used for the cultivation.

Identification of *S. bacillaris* MBTH40

The sequence of 16S rRNA gene of 40H were aligned in NCBI BLAST. As a result, 16S rDNA showed the significant homology with *Streptomyces bacillaris* strains (Sequence identity 98%). Therefore, 40H was identified as *Streptomyces bacillaris* and the strain was assigned as MBTH40 which stands for 40H from Microbial Biotechnology Laboratory. The partial sequence was submitted to NCBI and the accession number is MK966144 (Appendix). Neighbor joining tree was drawn based on the aligned sequence similarity of other species from NCBI depicting evolutionary relationships (Fig. 2).

Figure 1. Top agar overlay assay of *S. bacillaris* MBTH40

S. bacillaris MBTH40 was cultured in three different medium CM50, GTYB50, and YPM50. The size of inhibition zone appeared similar in GTYB50 and YPM50 agar, whereas CM50 agar has the smallest inhibition zone.

CM50 agar	GTYB50 agar	YPM50 agar
		

*The scale bar: 5 mm.

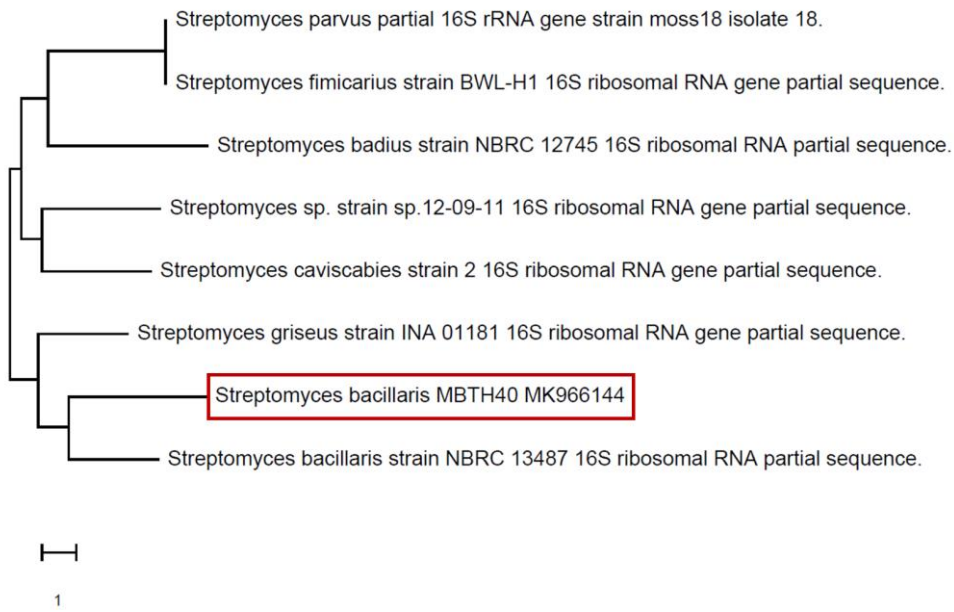
Table 1. MICs of culture extracts from different medium

	MIC vs. *S. aureus* (μg/mL)
GTYB50	16
YPM50	8
AMP*	0.06

* Positive control

Figure 2. Neighbor joining tree of *S. bacillaris* MBTH40

The neighbor joining tree depicts phylogenetic relationships of MBTH40. According to the sequence alignments of NCBI BLAST, MBTH40 has the highest homology with *S. bacillaris* species.


Streptomyces parvus partial 16S rRNA gene strain moss18 isolate 18.
Streptomyces fimicarius strain BWL-H1 16S ribosomal RNA gene partial sequence.
Streptomyces badius strain NBRC 12745 16S ribosomal RNA partial sequence.
Streptomyces sp. strain sp.12-09-11 16S ribosomal RNA gene partial sequence.
Streptomyces caviscabies strain 2 16S ribosomal RNA gene partial sequence.
Streptomyces griseus strain INA 01181 16S ribosomal RNA gene partial sequence.
Streptomyces bacillaris MBTH40 MK966144
Streptomyces bacillaris strain NBRC 13487 16S ribosomal RNA partial sequence.
1

Isolation of the compounds

Extraction and separation of bioactive compounds were conducted through serial partitioning (Fig. 3). By semi-preparative HPLC, fraction 1 (t_R: 16.9 min, 15.6 mg), 2 (t_R: 19.5 min, 18.7 mg), 3 (t_R: 19.7 min), 4 (t_R: 20.2 min, 50.3 mg), 5 (t_R: 21.5 min, 35.7 mg), 6 (t_R: 22.5 min), and 7 (t_R: 25 min, 18.3 mg) were collected (Fr. 1-Fr. 7), separately (Fig. 4). Since there were no major peak repeatedly appear in Fr. 3 and Fr. 6, they were not quantified. To elucidate the fraction possessing anti-staphlyococcal activity, MICs were determined (Table 2). Among seven fractions, Fr. 2, Fr. 5, Fr. 6 and Fr. 7 showed strong inhibitory activity against *S. aureus* at 2 μg/mL or lower concentration. For further purification, each fragment was subjected to another mobile settings in HPLC (Fig. S1). Peak 1 (4.2 mg) was flowed out from Fr. 1 at t_R11.4 min in 20% aq. ACN (+ 0.1% TFA) isocratic condition. With the same mobile setting, Peak 4-3 (3.7 mg) was flowed out from Fr. 4 at t_R 22.5 min. Peak 2 (5.8 mg) flowed out at t_R 12.5 min when Fr. 2 was purified through 30% aq. ACN (+ 0.1% TFA) isocratic condition. Peak 5 (20 mg) was appeared at t_R 16.0 min when Fr. 5 was subjected to gradient condition with 10% to 100% aq. ACN (+ 0.1% of TFA). These four compounds were purified as a single compound and then structurally analyzed.

Figure 3. Flow chart of extraction and separation of metabolites from *S. bacillaris* MBTH40

Filtrate of culture was extracted by several organic solvents and subjected to HPLC after all the organic solvents were removed.

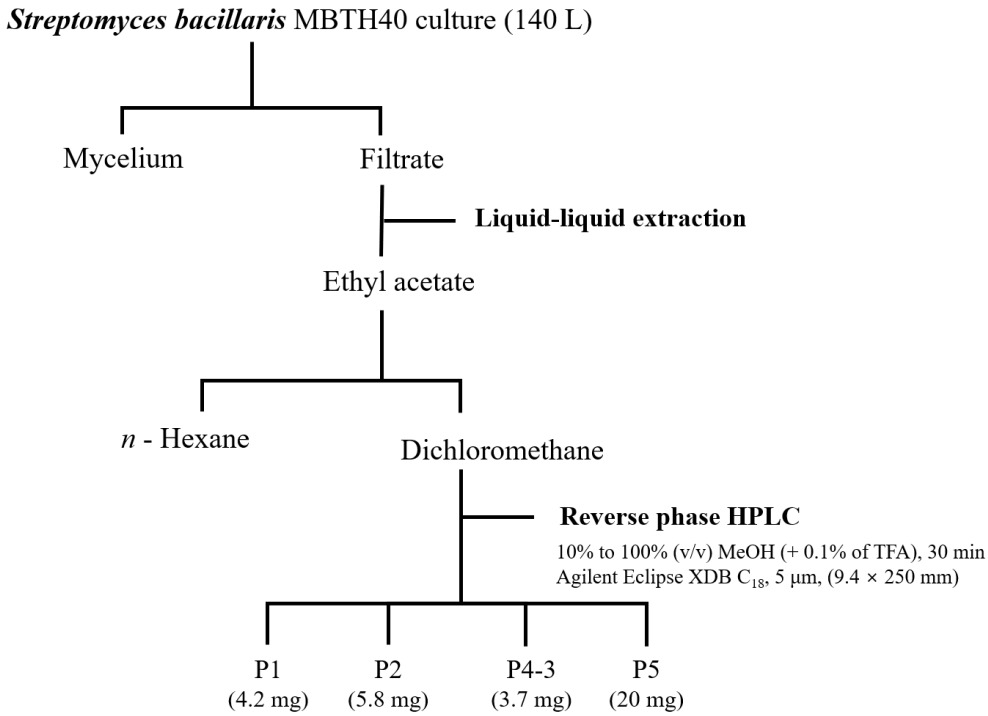

Streptomyces bacillaris MBTH40 culture (140 L)
Mycelium
Filtrate
Liquid-liquid extraction
Ethyl acetate
n - Hexane
Dichloromethane
Reverse phase HPLC
10% to 100% (v/v) MeOH (+ 0.1% of TFA), 30 min
Agilent Eclipse XDB C_{18}, 5 µm, (9.4 × 250 mm)
P1
(4.2 mg)
P2
(5.8 mg)
P4-3
(3.7 mg)
P5
(20 mg)

Figure 4. HPLC chromatogram of the extract of MBTH40 culture

Semi-preparative reverse-phase HPLC was conducted to separately collect seven fractions (Fr. 1 – Fr. 7).

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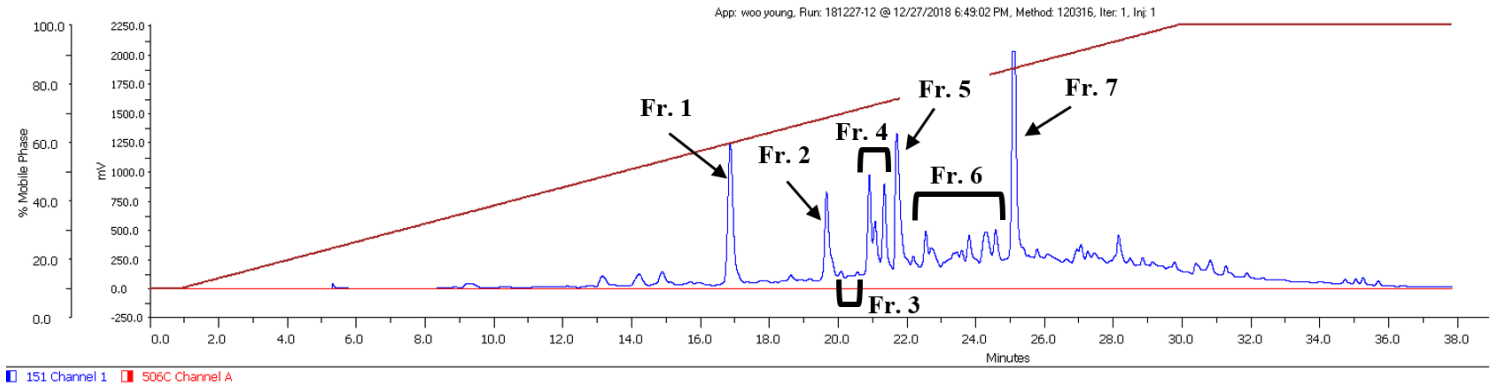

App: woo young, Run: 181227-12 @ 12/27/2018 6:49:02 PM, Method: 120316, Iter: 1, Inj: 1
% Mobile Phase
mV
Minutes
Fr. 1
Fr. 2
Fr. 3
Fr. 4
Fr. 5
Fr. 6
Fr. 7
151 Channel 1
506C Channel A

Table 2. Anti-staphylococcal activity of HPLC fractions

Samples.	MIC vs. *S. aureus* (μg/mL)
Fr. 1	128
Fr. 2	2
Fr. 3	> 128
Fr. 4	4
Fr. 5	≤ 0.25
Fr. 6	1
Fr. 7	≤ 0.25
AMP*	0.06

* Positive control

Structure elucidation

Fragmentation pattern and UV spectrum of P1 were observed (Fig. S2). UV absorption pattern of P1 showed similarity with the spectrum of 2, 4, 7-decatrienesaeure. According to the MS data, $[M+H]^+$ and $[M-H]^-$ of P1 were detected at m/z 112.2 and 110.9, respectively. Accompanied with ^1H NMR (Fig. S6) and ^{13}C NMR (Fig. S7) data, P1 was revealed as a pyrrol-2-carboxylic acid (CAS No. 634-97-9) (Hofle and Wolf, 1983). Molecular formula of pyrrol-2-carboxylic acid is $C_5H_5NO_2$ and its molecular weight is 111.1 g/mol.

MS spectrum and UV spectrum of P2 was also observed (Fig S3). According to the MS analysis, $[M+H]^+$ and $[M-H]^-$ of P2 were detected at m/z 474.2 and 472.2, respectively. Gathered with ^1H NMR (Fig. S8) and ^{13}C NMR (Fig. S9) data, P2 was revealed as a lactoquinomycin B (CAS No. 101342-94-3) which was first identified in 1985 (Okabe et al., 1985). Molecular formula of lactoquinomycin B is $C_{24}H_{27}NO_9$ and its molecular weight is 473.5 g/mol.

Fragmentation pattern and UV spectrum of P4-3 was observed (Fig. S4). UV absorption pattern of P4-3 was compared with the spectrum of terrain, however, they turned to be different compounds. $[M+H]^+$ and $[M-H]^-$ of P4-3 were detected at m/z 217.1 and 215.1, respectively. Molecular structure was confirmed via ^{13}H NMR spectrum (Fig. S10). P4-3 was matched to *N*-[2-(1H-indol-3yl)-2-oxoethyl]-acetamide also known as *N*-Acetyl-β-oxotryptamine (CAS No. 73053-91-5) (Chen et al., 1983). Its molecular formula is $C_{12}H_{12}N_2O_2$ and the molecular weight is 216.2 g/mol.

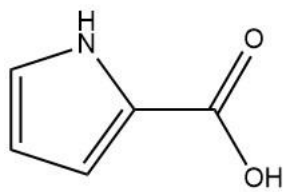
Molecular structure of P5 was confirmed by ^{1}H, ^{13}C NMR and 2D NMR (COSY, HMBC, HSQC) spectra (Fig. S11-15) and MS analyses (Fig. S5). The alignments were matched to lactoquinomycin A also known as medermycin (CAS No. 100100-36-5) (Okabe et al., 1985). Since the first discovery of lactoquinomycin A on 1976, position of angolosamine was revised from C8 to C6 (Leo et al., 2002; Williamson et al., 2002). Molecular formula of lactoquinomycin A is $C_{24}H_{27}NO_8$ and its molecular weight is 457.5 g/mol. In this paper, the structures were drawn with ChemDraw Software reflecting the revised model (Fig. 5).

Since P2 and P5 are analogues, δ_H and δ_C were compared, respectively (Table 3, 4). NMR assignments of P1 and P4-3 were presented individually (Table 5,6). Lactoquinomycin and its analogues are classified as benzoisochromane-quinones and pyranonaphthoquinone lactones, and so on (Ichinose et al., 2003; Ding et al., 2009).

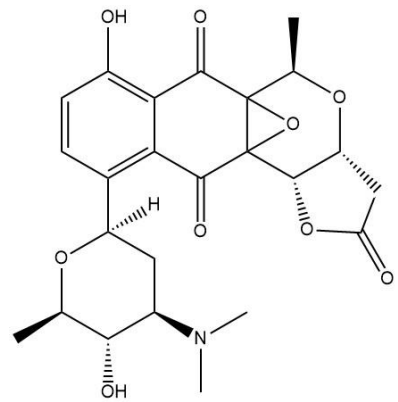
Figure 5. Structures of the compounds

(a) P1 is pyrrol-2-carboxylic acid; (b) P2 is lactoquinomycin B; (C) P4-3 is *N*-[2-(1H-indol-3yl)-2-oxoethyl]-acetamide also known as *N*-Acetyl-β-oxotryptamine; (d) P5 is lactoquinomycin A also known as medermycin.

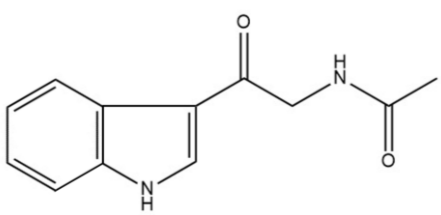
(a)



(b)



(c)



(d)

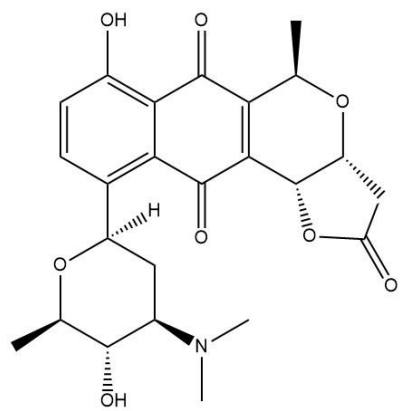
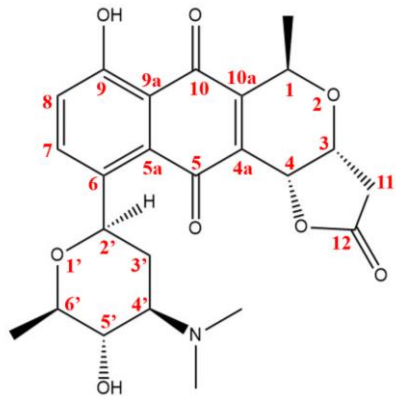
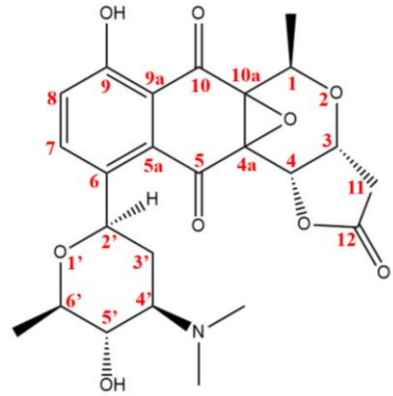


Table 3. Comparison of ^{1}H NMR data of LQM-A and LQM-B



LQM-A (P5)



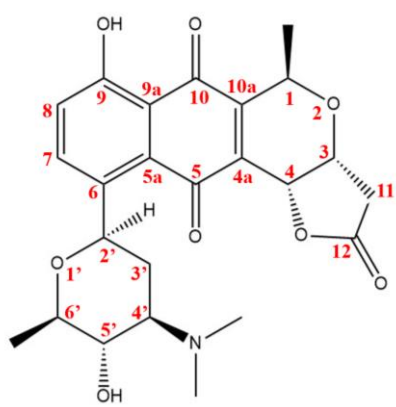
LQM-B (P2)

Position	LQM-A *	LQM-B *	Position	LQM-A *	LQM-B *
1-H	5.08	5.00	**2'-H**	4.77	4.71
3-H	4.61	4.61	**3'-H_{ax}**	1.68	1.67
4-H	5.32	5.48	**3'-H_{eq}**	2.53	2.53
6-H	7.70	7.69	**4'-H**	2.55	2.62
7-H	7.94	7.97	**5'-H**	3.15	2.91
11-H_1	2.54	2.43	**6'-H**	3.48	3.55
11-H_2	3.18	2.74	**4'-N $(CH_3)_2$**	2.83	2.79
1-CH_3	1.58	1.60	**6'-CH_3**	1.42	1.41

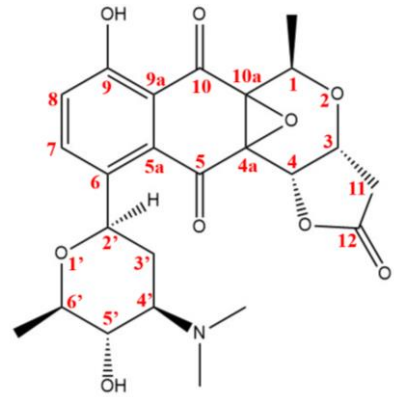
* δ_C relative to TMS

Samples were dissolved in methanol-d_4

Table 4. Comparison of ^{13}C NMR data of LQM-A and LQM-B



LQM-A (P5)



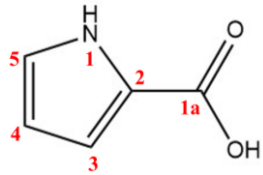
LQM-B (P2)

Position	LQM-A *	LQM-B *	Position	LQM-A *	LQM-B *
1	67.8	65.8	**10a**	151	65.9
3	68.2	66.5	**11**	37.5	36.4
4	70.9	71.2	**12**	177.3	177.3
4a	136.8	61.9	**1-CH_3**	18.3	14.8
5	182.9	188.8	**2'**	72.4	72.4
5a	132.3	132.3	**3'**	30.4	30.4
6	120	120.6	**4'**	68.6	68.5
7	134.8	135.2	**5'**	71.3	71.8
8	137.9	138	**6'**	78.65	78.6
9	158.9	159	**4'-N (CH_3)$_2$**	a 42.2	a 42.2
9a	116	115.4		b 37.7	b 37.5
10	190	196.6	**6'-CH_3**	18.6	18.2

* δ_C relative to TMS

Samples were dissolved in methanol-d_4

Table 5. ^{1}H and ^{13}C NMR assignments of P1

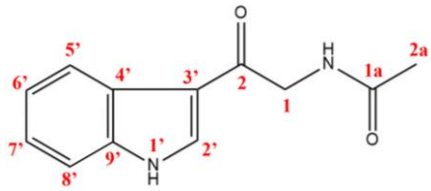


Position	δ_C *	δ_H *
2	122.87	
3	114.61	6.13
4	109.20	2.54
5	123.28	6.71
1a	161.9	

* Chemical shifts (ppm)

The sample was dissolved in methanol-d_4, and dimethyl sulfoxide-d_6 mixture

Table 6. ^{1}H NMR assignments of P4-3



Position	δ_H*
1	4.44
2'	7.23
5'	8.35
6	7.47
7'	7.49
8'	8.16
2a	1.91

* Chemical shifts (ppm)

The sample was dissolved in methanol-d_4, and dimethyl sulfoxide-d_6 mixture

Antibacterial and antifungal activity test

While P1 and P4-3 did not exhibit interesting activity, P2 and P5 exerted inhibitory activity (Table 6). Stronger activity of P5 relative to P2 is presumably due to the absence of epoxide moiety. Generally, P5 seems more effective against gram-positive than gram-negative bacteria, but it also inhibited *S. enterica* displaying lower MIC than that of reference compounds, ampicillin and tetracycline. Given the potent anti-staphylococcal activity of P2 and P5, MIC against MRSA strains were investigated as well (Table 7). Most of the tested strains were inhibited by P5 more efficiently than the reference compounds. Besides, P2 showed moderate inhibition with MIC range 8 to 16 μg/mL

None of the four compounds showed antifungal activity within 128 μg/mL concentration (Table 8).

Table 7. Antibacterial activity assay

		MICs (μg/mL)					
		P1	**P2**	**P4-3**	**P5**	**AMP***	**TET***
Gram (+)	[a]***Staphylococcus aureus***	> 128	2	> 128	0.13	0.13	0.25
	[b]***Enterococcus faecalis***	> 128	64	> 128	1	0.25	0.5
	[c]***Enterococcus faecium***	> 128	32	> 128	0.5	0.5	0.25
Gram (-)	[d]***Klebsiella pneumoniae***	> 128	> 128	> 128	> 128	> 128	0.25
	[e]***Salmonella enterica***	64	1	> 128	0.015	0.25	2
	[f]***Escherichia coli***	> 128	> 128	> 128	16	16	0.5

* Positive controls

[a] *Staphylococcus aureus* ATCC 25923, [b] *Enterococcus faecalis* ATCC 19433

[c] *Enterococcus faecium* ATCC 19434, [d] *Klebsiella pneumoniae* ATCC 10031

[e] *Salmonella enterica* ATCC 14028, [f] *Escherichia coli* ATCC 25922

Table 8. MICs against MRSA strains

MRSA strains	MICs (µg/mL)			
	P2 (LQM-B)	P5 (LQM-A)	CIP*	VAN*
ATCC 43300	8	0.06	0.5	0.5
ATCC 700787	16	0.25	0.5	0.5
ATCC 700788	16	0.25	32	1
CCARM 3089	8	0.25	128	0.5
CCARM 3090	16	0.5	128	0.06
CCARM 3634	8	0.25	128	0.13
CCARM 3635	8	0.25	128	0.5
CCARM 33591	8	0.25	0.5	0.5

* Positive controls

Table 9. Antifungal activity assay

	MICs (μg/mL)				
	P1	**P2**	**P4-3**	**P5**	**AMB***
[a] ***Candida albicans***	> 128	> 128	> 128	> 128	0.25
[b] ***Aspergillus fumigatus***	> 128	> 128	> 128	> 128	1
[c] ***Trichophyton rubrum***	> 128	> 128	> 128	> 128	0.25
[d] ***Trichophyton mentagrophytes***	> 128	> 128	> 128	> 128	1

***** Positive control

[a] *Candida albicans* ATCC 10231, [b] *Aspergillus fumigatus* HIC 6094

[c] *Trichophyton rubrum* NBRC 9185, [d] *Trichophyton mentagrophytes* IFM 40996

ICL inhibitory assay

Beside the direct growth inhibitory activity of the compounds, enzyme inhibition assay was performed to evaluate the potential as an anti-virulence molecule. To examine ICL inhibitory activity of the isolated metabolites, IC_{50} values were measured (Table 9). Only P2 exhibited mild inhibition with IC_{50} 40.63 μg/mL, whereas other substances showed no inhibition. However, the activity of P2 was not as strong as a reference compound, 3-nitropropionate, of which IC_{50} value is 2.71 μg/mL.

Table 10. IC_{50} toward ICL of the four compounds

Compounds	IC_{50} (μg/mL)
P1	> 128
P2	40.63
P4-3	> 128
P5	> 128
3-nitropropionate*	2.71

* Positive control

Discussion

In this experiment, actinobacteria from relatively untapped source was chosen. From sediment of the Pacific Ocean, *S. bacillaris* MBTH40 was isolated. Fermented broth of MBTH40 was partitioned and purified, followed by the structural studies and bioactivity assays. All the isolated compounds were identified to be the known. To direct biosynthesis of unannotated metabolites, there are other directions to explore. For instance, altering precursors, affecting tailoring processes by genetic engineering, and organism-independent strategy may increase the probability to yield novel products (Olano et al., 2009; Katz et al., 2016; Janata et al., 2018). Furthermore, approaches involving signaling molecules, co-culturing and other methods are also being studied to attain unprecedented bioactive compounds (Antoraz et al., 2015). Above all, accompanying dereplication process would maximize the probability of discovering unknown substances (Ito and Masubuchi, 2014).

LQM-A (P5) was a major secondary metabolite produced YPM50 grown *S. bacillaris* MBTH40. Not only the amount, but potencies of bioactivities were also highest among four isolated compounds. Soon after the first discovery of medermycin from *Streptomyces* sp. K73 in 1976 (Takano et al., 1976), independently characterized LQM-A, which has both anticancer and antibacterial activities, was found to be identical with medermycin (Tanaka et al., 1985). There has been nine medermycin analogues (lactoquinomycin A-D, mederrhodin A, B, menoxymycin A, B and MDN-0171) reported so far (Takano et al., 1976; Okabe et al, 1986; Hayakawa

et al., 1994; Omura et al, 1986; Lacret et al., 2018; Zhou et al., 2019). On 1988, the mechanism of medermycin as an antibiotic was investigated through experiments covering macromolecule syntheses, and the level of ATP, NADH and superoxide (Nomoto et al., 1988). However, they concluded the paper that the exact cause of cytotoxicity remains to be elucidated. On the other hand, the mode of anticancer activity was also investigated recently. The researchers explained that abrogation of catalysis of AKT, a protein kinase crucial for the proliferation of tumor cells, is the mode of action (Toral-Barza et al., 2007; Salaski et al., 2009). Still, further examinations are required to unravel the antibacterial mechanism of medermycin. On 2003, medermycin biosynthetic gene cluster, *med* cluster, was cloned from *Streptomyces* sp. AM-7161 and expressed in heterologous host, *Streptomyces coelicolor* CH999; (Ichinose et al., 2003). As for the structural researches, revised structure of glycosylated naphthoquinones including lactoquinomycin A and B were reported in 2002. Position of the angolosamine was corrected to be attached *para* to a phenolic group, not *ortho*, based on the total syntheses results and NMR spectra (Leo et al., 2002; Williamson et al., 2002).

Although pyrrol-2-carboxylic acid and *N*-[2-(1H-indol-3yl)-2-oxoethyl]-acetamide did not exert bioactivities in this study, their activities were examined in other studies. *N*-[2-(1H-indol-3yl)-2-oxoethyl]-acetamide, also known as *N*-acetyl-β-oxotryptamine was reported to have an anti-trypanosomal activity against *Trypanosoma cruzi,* parasite causing tropical disease, with IC_{50} 19.4 μM (Martinez-Luis et al., 2012). Similarly, pyrrol-2-carboxylic acid, which was a P1 in this experiment, was observed to inhibit *Phytophthora capsici,* a plant pathogenic oomycete, with MIC value of 4 μg/mL (Nguyen et al., 2015). Both pathogens belong

to domain eukaryota.

Beside the four compounds whose structure was unraveled, there were other fragments possessing anti-staphylococcal activity. Purification of the unpurified fragments to a single compound may be possible under conditions not attempted in this study. In addition, further studies involving structure-activity relationship studies, genetic approaches and non-traditional strategies would be worthwhile.

It is well recognized that the extraordinary diversity of microbial derivatives provided benefits to pharmaceuticals and agriculturals (Davies, 2007). Within the *Streptomyces* genus alone produce numerous small molecules (less than 1 kDa) with diverse functions and structural complexity (Berdy, 2012; Sivalingam et al., 2019). They are biologically active effecting biochemical reactions with high specificity (Davies, 2007). Exploration of *Streptomyces* inhabiting in deep sea has been steadily increasing since this environment possess the richest biodiversity (Skropeta, 2008). Corresponding target studies (e. g. microbial communication, pathogenicity, and host-pathogen interaction) should be comprised with the inhibitor discovery (Maddocks, 2016). Researches on the deep-sea derived *Streptomyces*, with the modern biotechnological approaches and comprehensive understanding of targets, is necessary to meet the urgent need of the antibacterial agents.

In conclusion, marine-derived *S. bacillaris* MBTH40 cultivated in YPM50 media was found to produce pyranonaphthoquinones, LQM-A and LQM-B. These compounds exhibited inhibitory bioactivities against various pathogenic bacteria *in vitro*. Especially LQM-A was appeared as a major secondary metabolite of this species. Additionally, LQM-B displayed mild inhibition of ICL enzyme activity

implying the potential as an anti-virulence molecule. Although the other two compounds were not observed to be bioactive in this research, their activities against eukaryotic micro-organisms were reported (Martinez-Luis et al., 2012; Nguyen et al., 2015).

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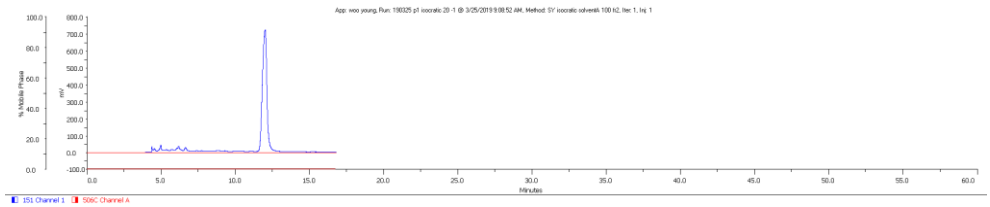
Supplementary Materials

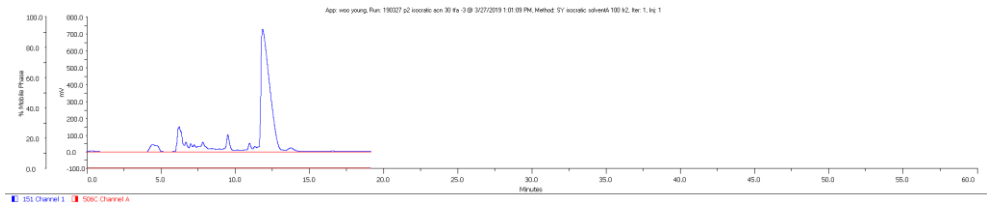
Supplementary Figure 1. Purifying conditions for each fraction

(a) P1 was purified in isocratic condition with the solvent aq. 20% ACN including 0.1% TFA; (b) P2 was purified in isocratic condition with the solvent aq. 30% ACN including 0.1% TFA; (c) P4-3 was purified in isocratic condition with the solvent aq. 20% ACN including 0.1% TFA; (d) P5 was purified in gradient condition from aq. 10% to 100% ACN including 0.1% TFA in 30 min.

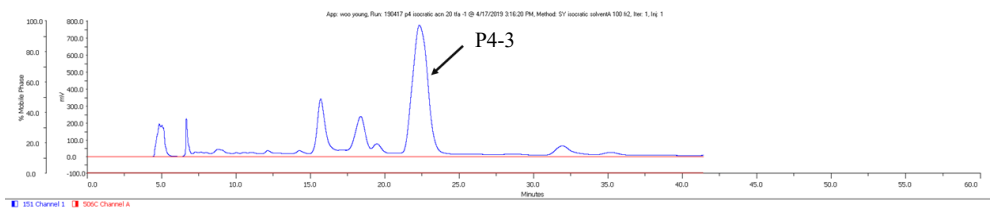
(a)



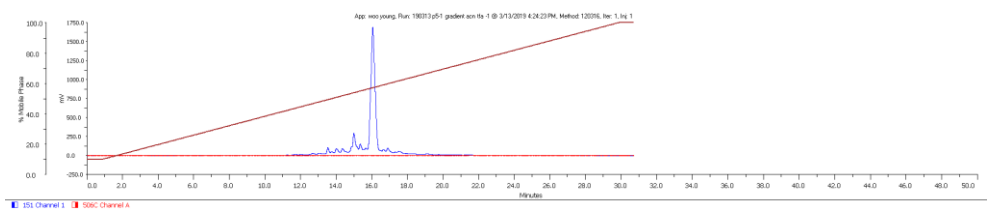
(b)



(c)



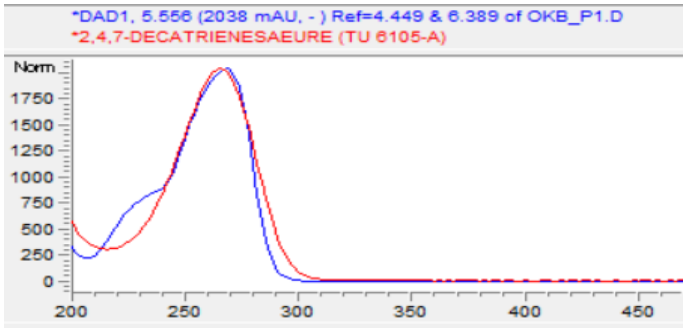
(d)



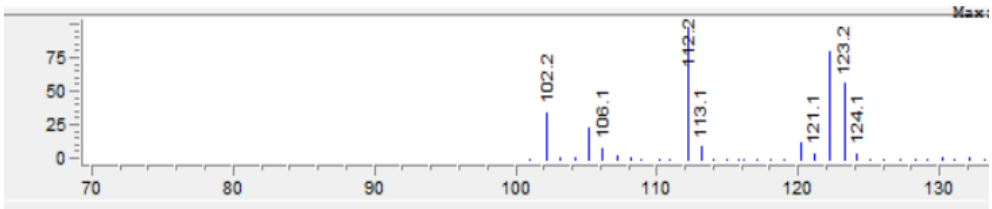
Supplementary Figure 2. UV spectrum and mass spectra of P1

(a) UV absorption pattern of P1 (blue line) was compared with the spectrum of 2, 4, 7-decatrienesaeure (red line). The spectrum showed λ_{max} at 270 nm; (b) Positive ion mode, $[M+H]^+$ = 112.2; (c) Negative ion mode, $[M-H]^-$ = 110.9

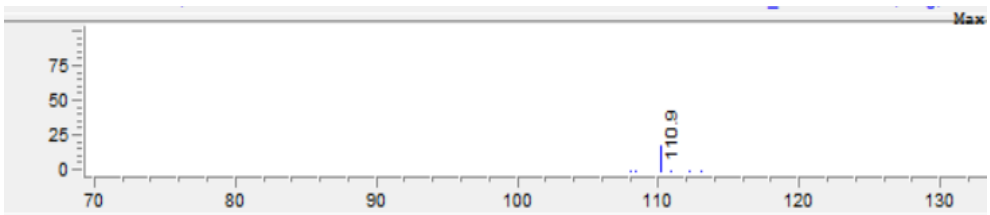
(a)


*DAD1, 5.556 (2038 mAU, -) Ref=4.449 & 6.389 of OKB_P1.D
*2,4,7-DECATRIENESAEURE (TU 6105-A)
Norm.
1750
1500
1250
1000
750
500
250
0
200
250
300
350
400
450

(b)


75
50
25
0
102.2
106.1
112.2
113.1
121.1
123.2
124.1
70
80
90
100
110
120
130

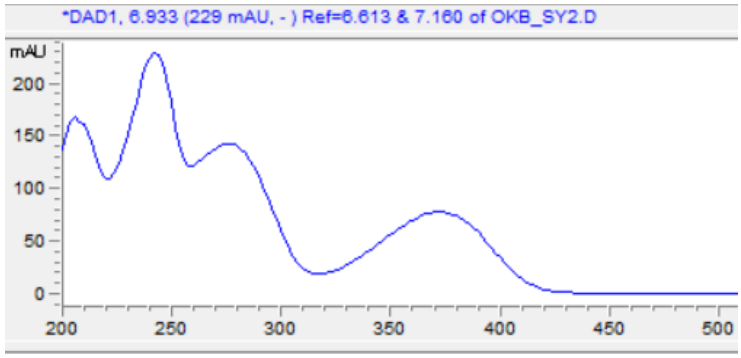
(c)


75
50
25
0
110.9
70
80
90
100
110
120
130

Supplementary Figure 3. UV spectrum and mass spectra of P2

(a) UV absorption pattern of P2 showed λ_{max} at 242 nm; (b) Positive ion mode, $[M+H]^+$ = 474.2; (c) Negative ion mode, $[M-H]^-$ = 472.2

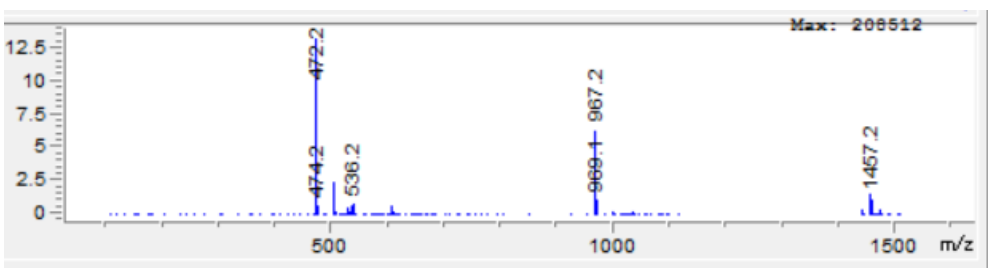
(a)


*DAD1, 6.933 (229 mAU, -) Ref=6.613 & 7.160 of OKB_SY2.D
mAU
200
150
100
50
0
200
250
300
350
400
450
500

(b)


Max: 1.55597e+006
474.2
476.2
80
60
40
20
0
500
1000
1500
m/z

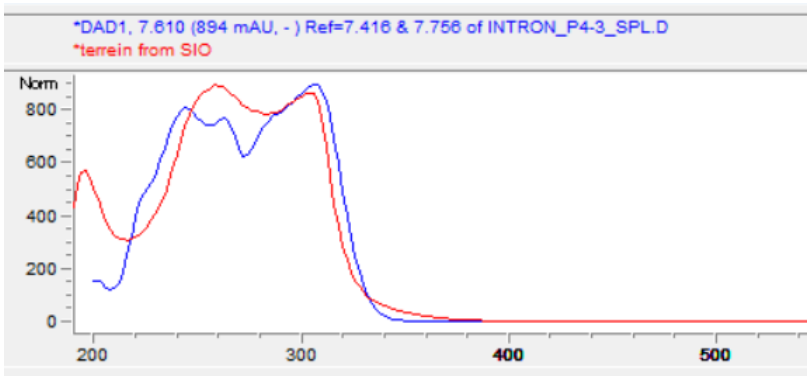
(c)


Max: 208512
472.2
474.2
536.2
967.2
969.1
1457.2
12.5
10
7.5
5
2.5
0
500
1000
1500
m/z

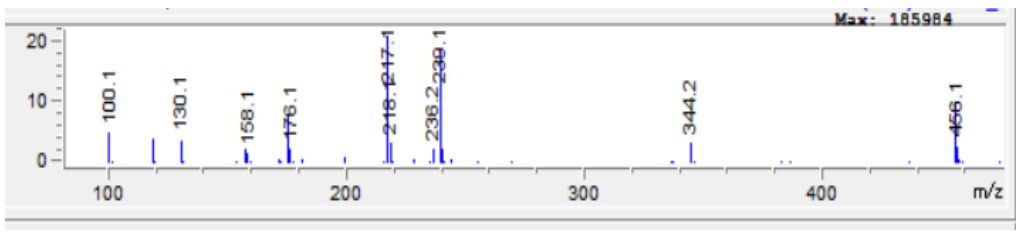
Supplementary Figure 4. UV spectrum and mass spectra of P4-3

(a) UV absorption pattern of P4-3 (blue line) was compared with the spectrum of terrein (red line) which was a putative molecule. The spectrum showed λ_{max} of P4-3 is 308 nm; (b) Positive ion mode, $[M+H]^+$ = 217.1; (c) Negative ion mode, $[M-H]^-$ = 215.1

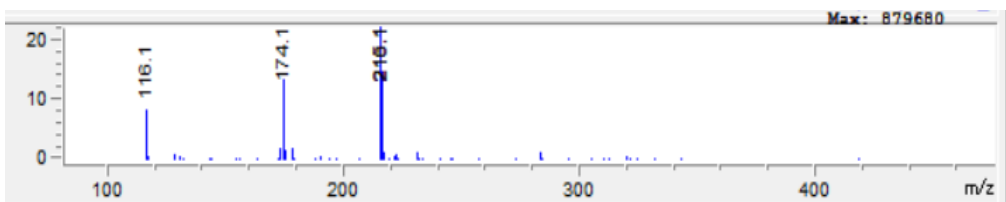
(a)


*DAD1, 7.610 (894 mAU, -) Ref=7.416 & 7.756 of INTRON_P4-3_SPL.D
*terrein from SIO
Norm
800
600
400
200
0
200
300
400
500

(b)


Max: 185984
20
10
0
100.1
130.1
158.1
176.1
218.2
217.1
236.2
239.1
344.2
456.1
100
200
300
400
m/z

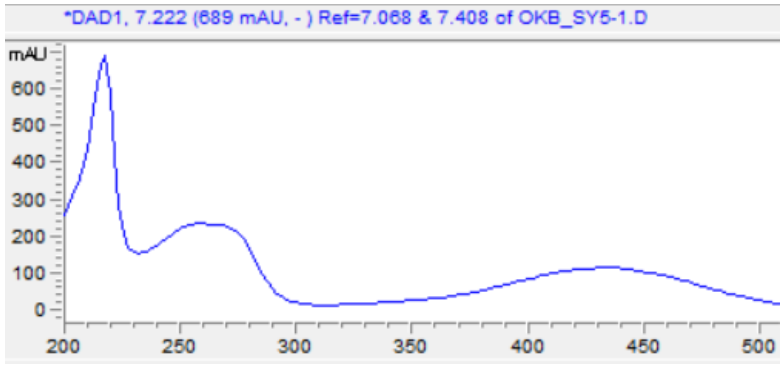
(c)


Max: 879680
20
10
0
116.1
174.1
218.1
100
200
300
400
m/z

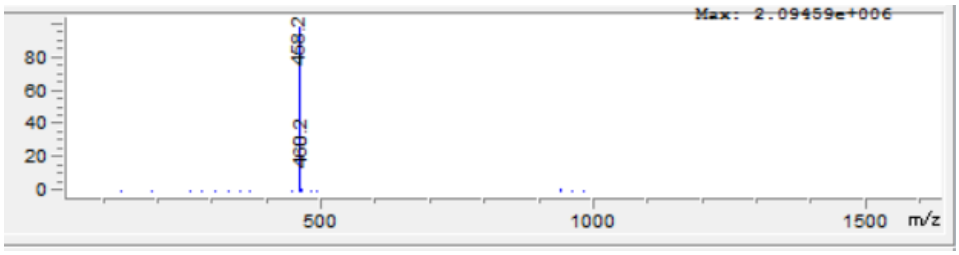
Supplementary Figure 5. UV spectrum and mass spectra of P5

(a) UV absorption pattern of P5 showed λ_{max} at 219 nm; (b) Positive ion mode, $[M+H]^+$= 458.2; (c) Negative ion mode, $[M-H]^-$ = 456.1

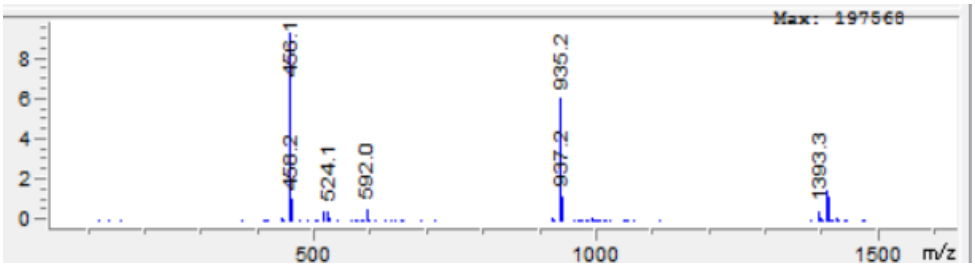
(a)


*DAD1, 7.222 (689 mAU, -) Ref=7.068 & 7.408 of OKB_SY5-1.D
mAU
600
500
400
300
200
100
0
200
250
300
350
400
450
500

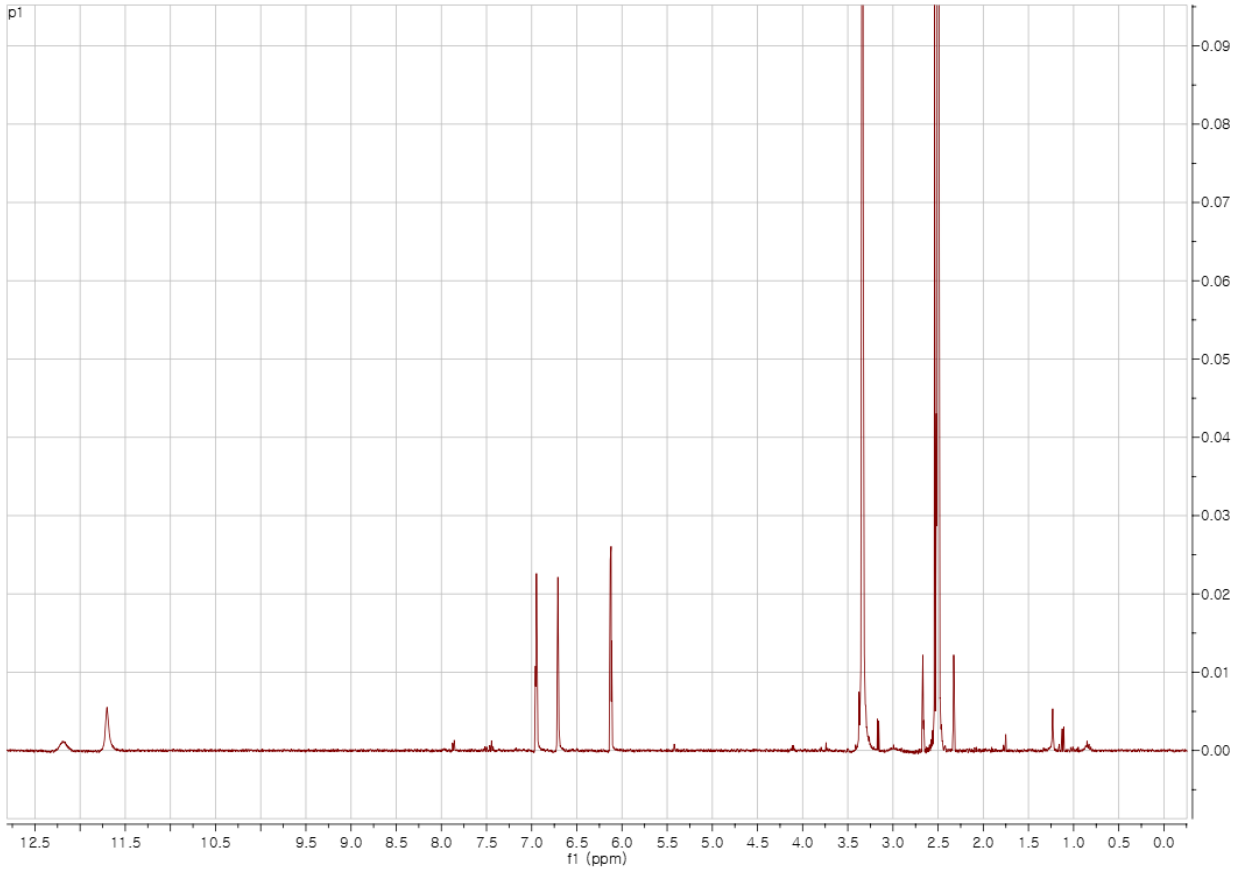
(b)


Max: 2.09459e+006
458.2
460.2
80
60
40
20
0
500
1000
1500
m/z

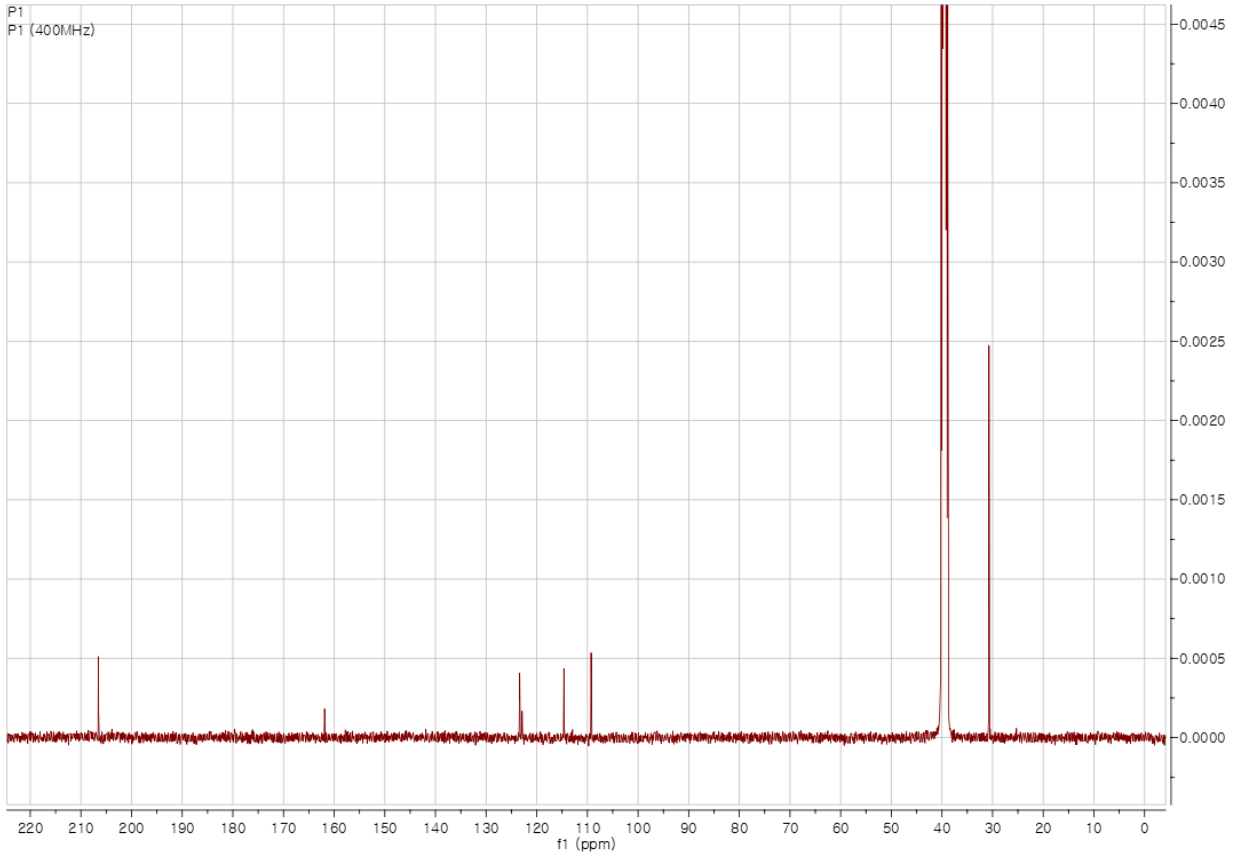
(c)


Max: 197568
456.1
458.2
524.1
592.0
935.2
937.2
1393.3
8
6
4
2
0
500
1000
1500
m/z

Supplementary Figure 6. ^{1}H NMR spectrum of P1 in methanol-d_4 and dimethyl sulfoxide-d_6

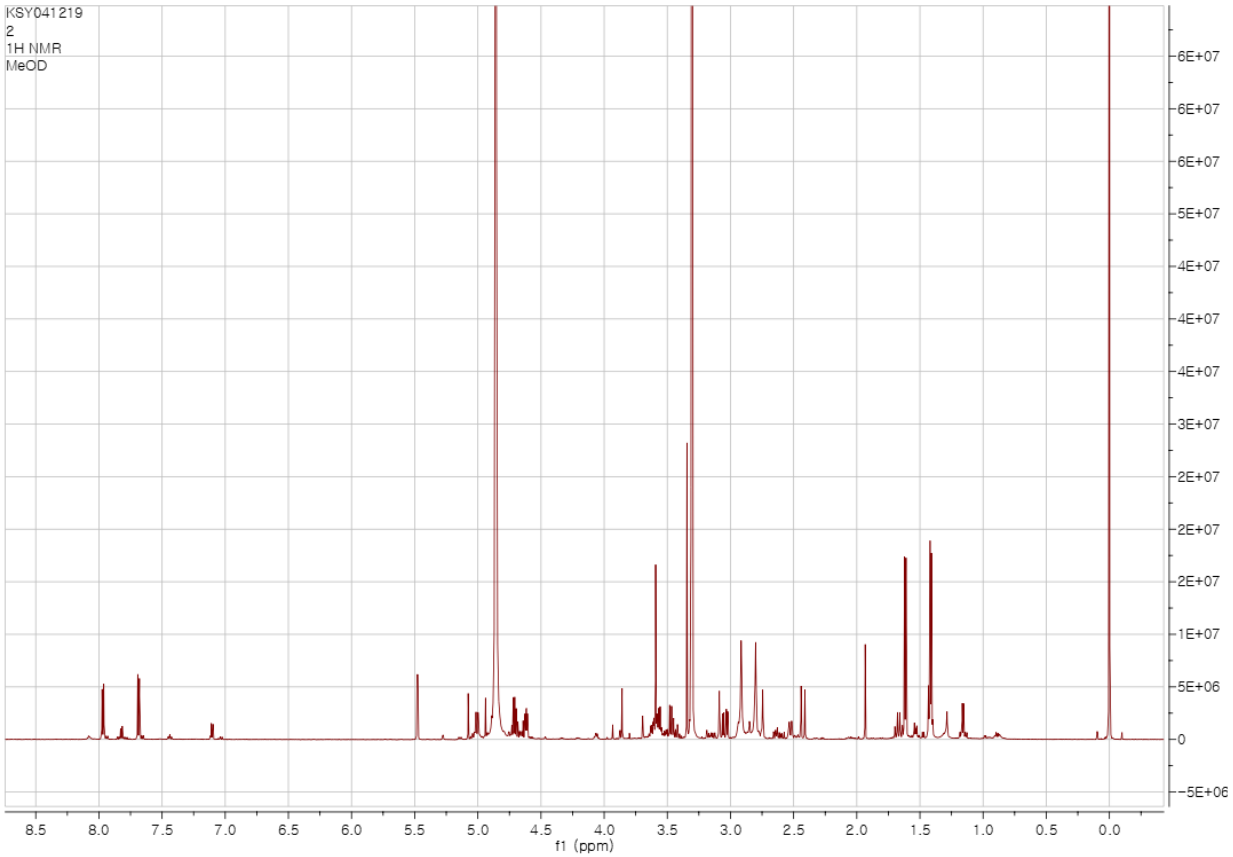


Supplementary Figure 7. ^{13}C NMR spectrum of P1 in dimethyl sulfoxide-d_6

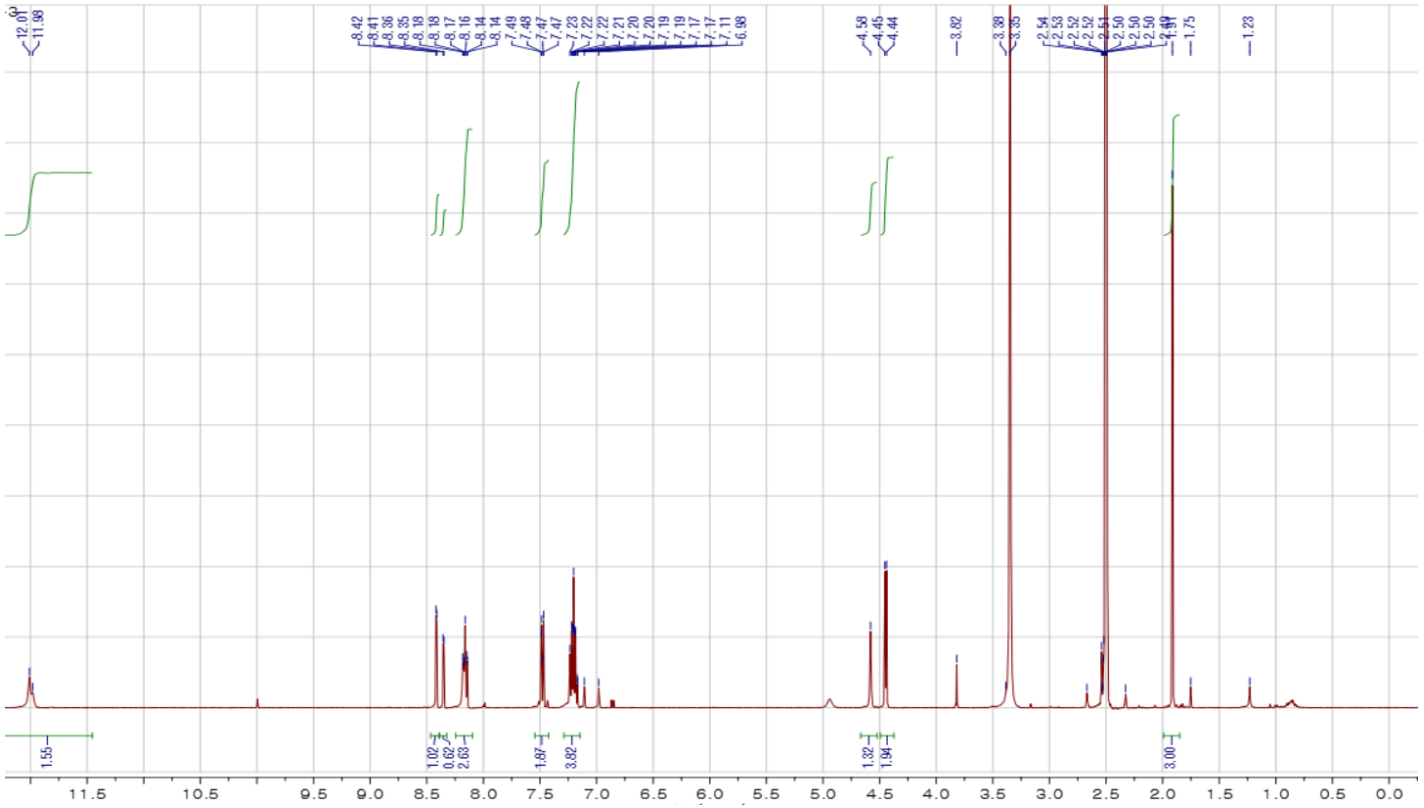


Supplementary Figure 8. ^{13}C NMR spectrum of P2 in methanol-d_4

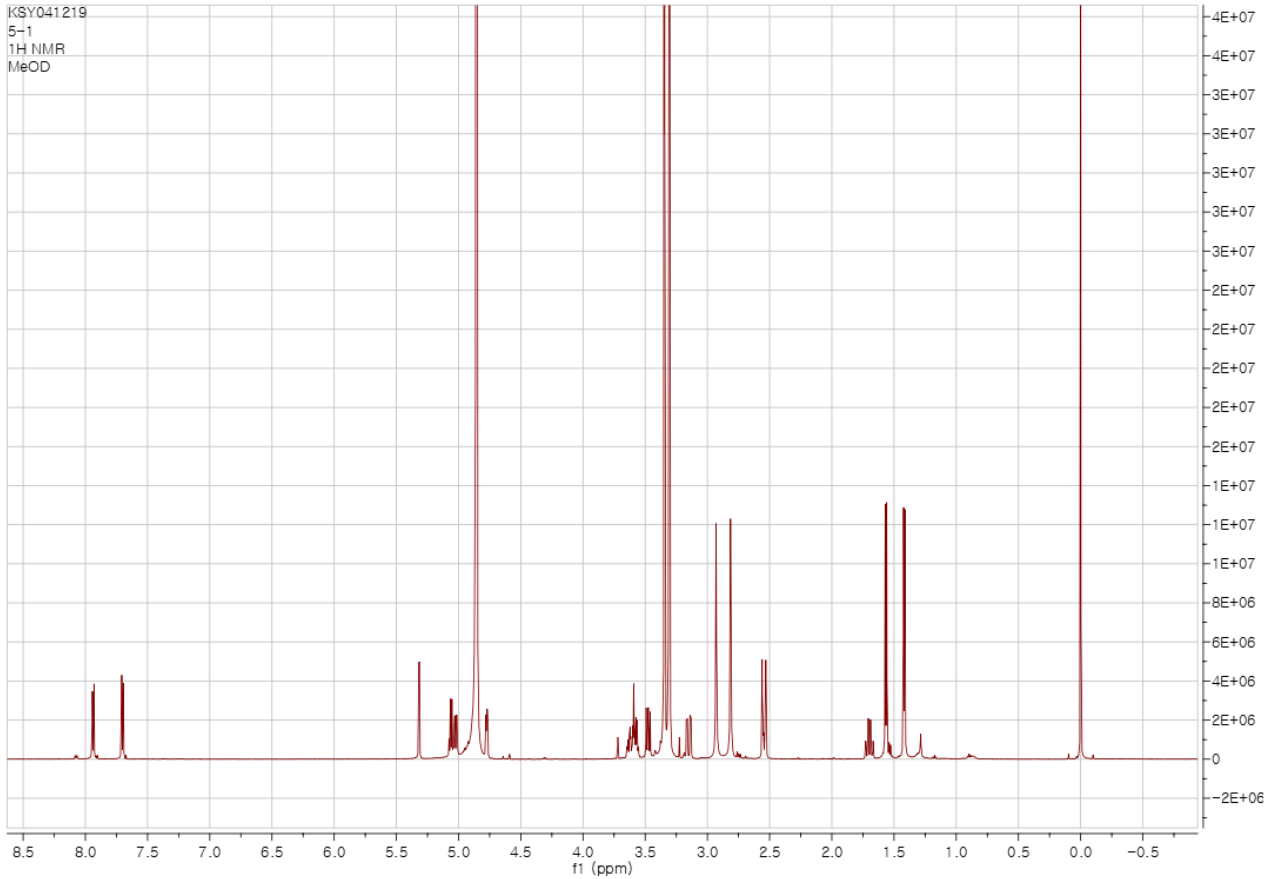
Supplementary Figure 9. ^{1}H NMR spectrum of P2 in **methanol-d$_4$**



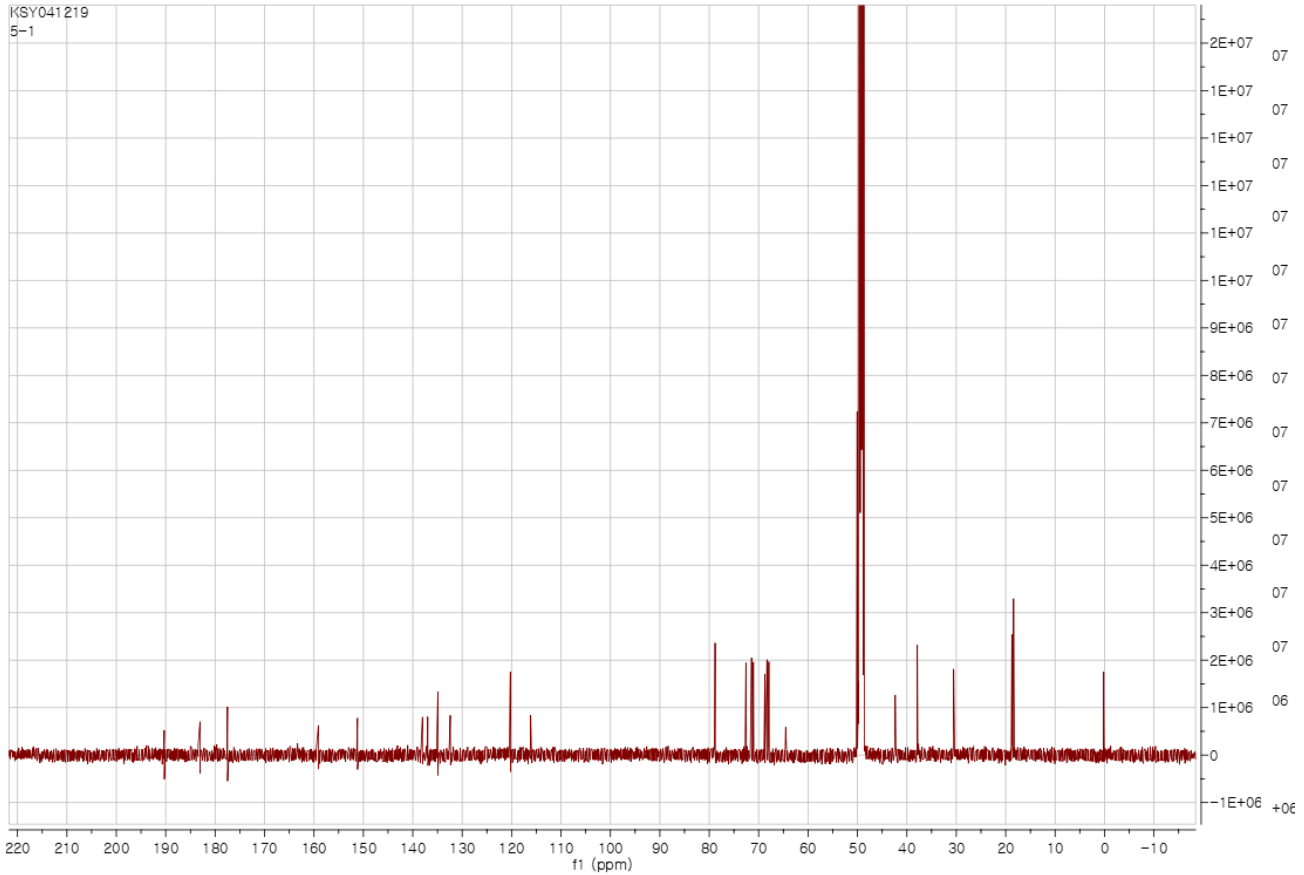
Supplementary Figure 10. ^{1}H NMR spectrum of P4-3 in methanol-d_4 and dimethyl sulfoxide-d_6



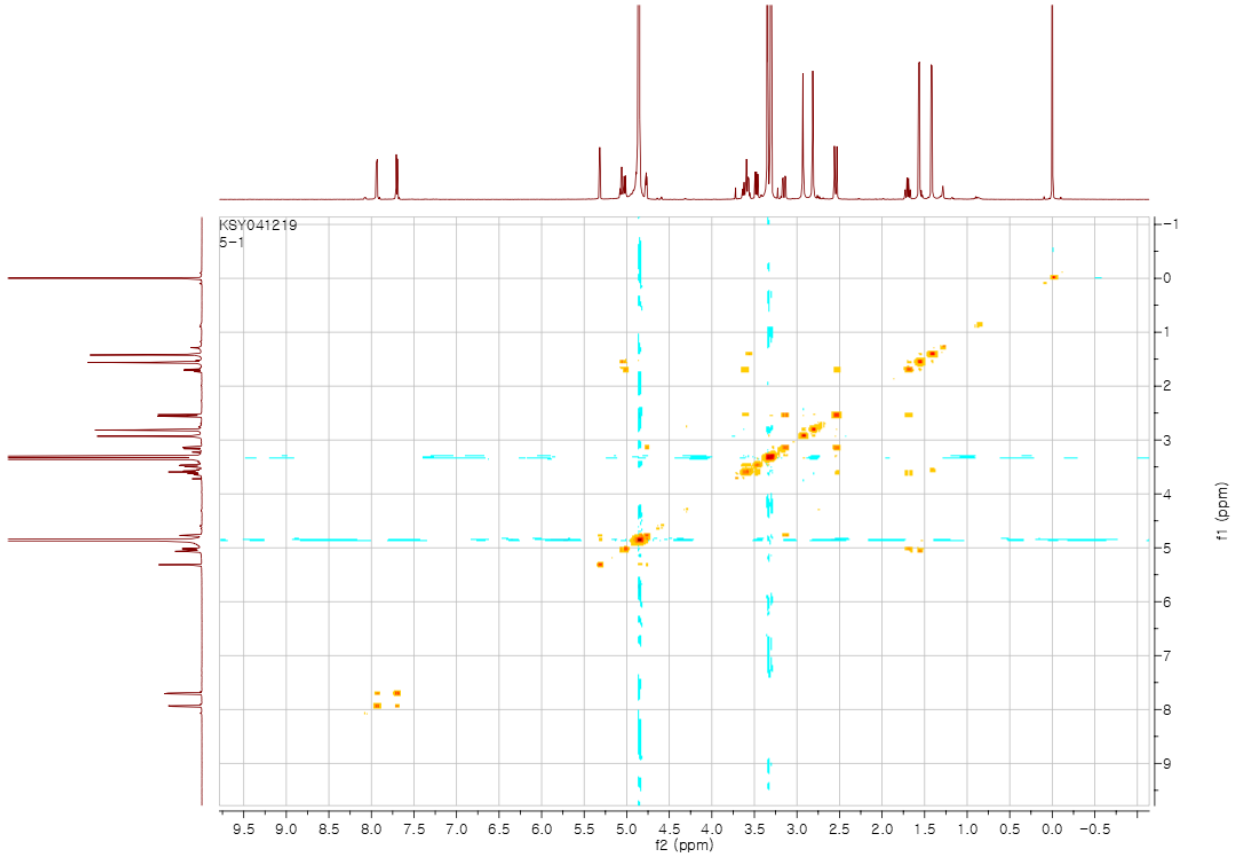
Supplementary Figure 11. ^{1}H NMR spectrum of P5 in methanol-d_4



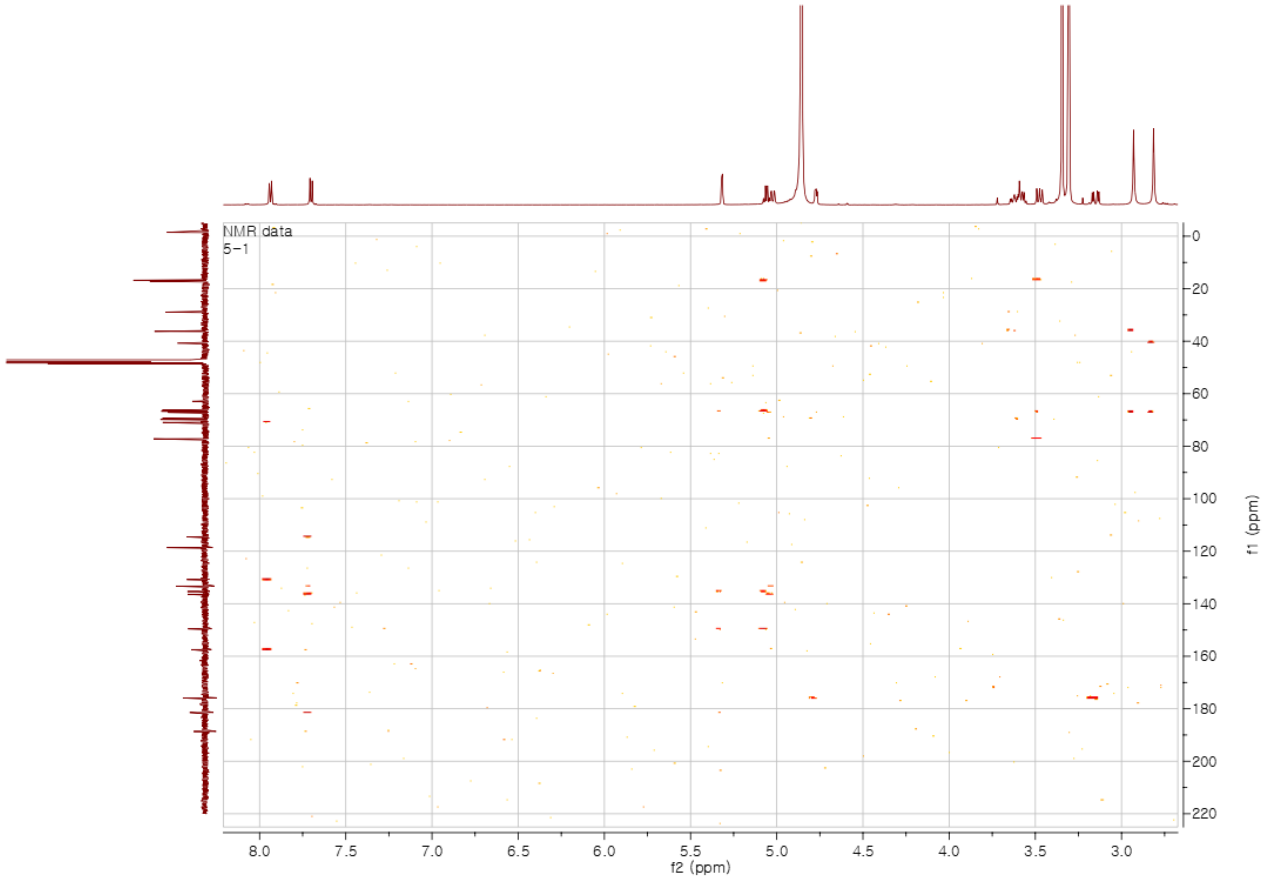
Supplementary Figure 12. ^{13}C NMR spectrum of P5 in methanol-d_4



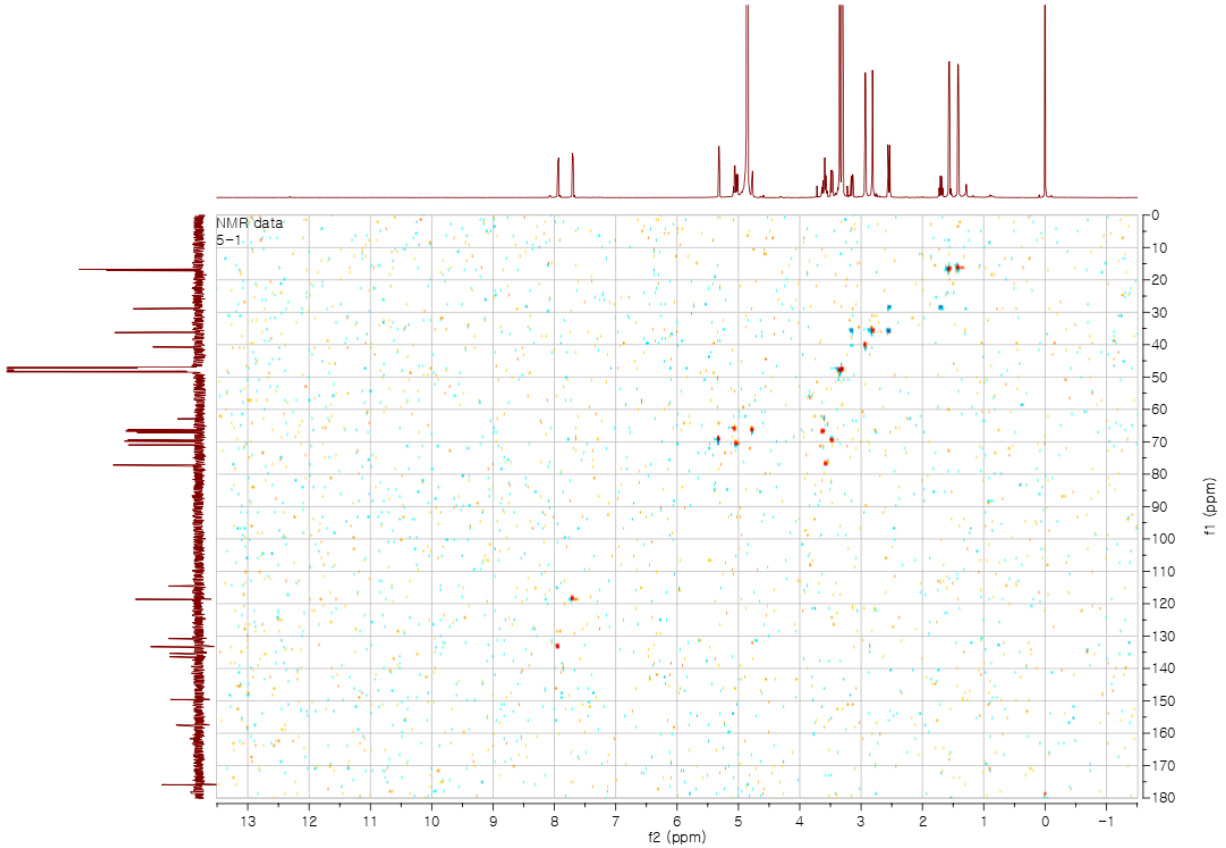
Supplementary Figure 13. ^{1}H-^{1}H COSY spectrum of P5 in methanol-d_4



Supplementary Figure 14. HMBC spectrum of P5 in **methanol-d_4**



Supplementary Figure 15. HSQC spectrum of P5 in **methanol-d_4**



Appendix

16S ribosomal RNA gene partial sequence of *Streptomyces bacillaris* MBTH40

CGCCAAGTGGCGGGGTGCTTACACATGCAGTCGAACGATGAAGCCGCTTCGGTGGTGGATTAGTGGC

GAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAA

TACCGGATAATACTCTGTTCCGCATGGAACGGGGTTGAAAGCTCCGGCGGTGAAGGATGAGCCCGCG

GCCTATCAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCG

ACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC

AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTT

CAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGC

CGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGT

CACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGG

TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGA

AGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

CCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAG

CTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG

GCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGAC

ATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACAGGTGGTGCATGGCTGTCGT

CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCA

TGCCTTTCGGGGTGATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA

CGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCG

ATGCCGTGAGGCGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCC

CATGAAGTCGGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTAC

ACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG

GAGCTGTCGAAGGTGGGACTGGCGATGGACGAAGTCGTAACAAGAGCTTCAGGGGG

Abstract in Korean

서울대학교 대학원

농생명공학부 응용생명화학전공

강 새 연

방선균은 약리학적 가치를 갖는 다양한 이차 대사산물을 생산하며 인간에게 유용한 물질들을 제공하여 왔다. 특히 상대적으로 연구가 덜 이루어진 해양 방선균은 다양한 압력, 온도, 빛과 산소 조건 등 토양과는 다른 환경 조건에 서식하기에 이에 기인한 특성의 일원으로 신물질 생산 대상으로서 주목받고 있다. 한국해양과학기술원으로부터 제공받은 태평양 해저 토양으로부터 분리한 406종의 방선균 중 *Staphylococcus aureus* 라는 병원균에 저해 활성을 보이는 40H를 선정하였다. 이후 16S ribosomal RNA 유전자 분석을 통하여 *Streptomyces bacillaris* 로 동정하였고 균주 이름을 MBTH40으로 명명하였다. 액체-액체 추출 및 HPLC를 통하여 대사산물을 분리하였고 분광학적 데이터를 얻어 총 네 가지 물질의 구조를 규명하였다. 모든 물질들은 이미 발견된 화합물들이었다. 각 물질의 항세균, 항진균, 그리고 isocitrate lyase (ICL) 효소 저해 활성여부를 실험한 결과 lactoquinomycin A가 methicillin-resistant *Staphylococcus aureus*

(MRSA) 균주를 포함한 병원성 세균에 대하여 저해활성을 가장 강하게 나타냈다. 항진균 활성을 나타낸 물질은 없었으며 lactoquinomycin B 가 약하게 ICL의 활성을 저해하는 것으로 나타났다.

주요어: 해양 방선균, *Streptomyces bacillaris,* 이차 대사산물, 항균 활성 물질, 메티실린 저항성 *Staphylococcus aureus*

학 번: 2018-27278

Acknowledgements

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