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Actinomycetes of rhizosphere soil producing antibacterial compounds against Urinary Tract Infection bacteria

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Abstract. *Apsari PP, Budiarti S, Wahyudi AT. 2019. Actinomycetes of rhizosphere soil producing antibacterial compounds against Urinary Tract Infection bacteria. Biodiversitas 20: 1259-1265.* Based on the ability of actinomycetes as an antibacterial compounds producer and the need of finding novel antibacterial compounds, this study aims to look for antibacterial compounds from rhizosphere actinomycetes against bacteria in urine of UTI patients (*Escherichia coli*, *Citrobacter braakii*, *Acinetobacter calcoaceticus*, and *Klebsiella pneumoniae*). The screening of 21 actinomycetes was conducted based on the paper disc method. Potential actinomycetes that inhibited test bacteria were, then cultured in liquid medium and the supernatant was tested to six test bacteria. Then, the supernatant was extracted using ethyl acetate and crude extract from extraction process was tested to test bacteria. Afterward, the crude extract was scanned for bioactive compounds by GC-MS. Potential actinomycetes were identified by 16S rRNA gene to reveal the species. The screening results showed that ARJ 16, 24, and 36 had a wider inhibition zone than others. All of them showed that the supernatant and the crude extract could inhibit UTI's bacteria. The highest abundance of bioactive compound of crude extract was found in Propane, 1,2-dichloro, n-Hexadecanoic acid, and Carbonochloridic acid, 2-chloroethyl ester, respectively. Identification of potential actinomycetes based on 16S rRNA gene showed that ARJ 16 and ARJ 24 were highly similar to *Streptomyces* sp. in 99% and ARJ 36 was similar to *Streptomyces tendae* in 98%.

Keywords: Actinomycetes, antibacterials, GCMS, Urinary Tract Infection

INTRODUCTION

The number of Multi-Drug Resistance (MDR) cases all over the world are still high and increasing each year (WHO 2018). There are some reports regarding MDR evidence, indicating that the rate of bacteria experienced MDR to cephalosporin significantly increased from 9.6% to 12% between 2011 and 2014 in Europe (ECDC 2015). In Southeast Asia, the resistance rate of bacteria to carbapenem (64.91%) and multidrug (58.51%) showed high percentage (Teerawattanapong et al. 2018). On the other hand, most *E. coli* (64.9%) from UTI patients in Nepal was reported to have resistance against antibiotics (Parajuli et al. 2017). *Klebsiella pneumoniae* also showed significant resistance to several antibiotic classes by multi mechanisms in worldwide (Navon-Venezia et al. 2017). *Acinetobacter* is one of nosocomial bacteria in America Latin, resistant to carbapenem class antibiotic in high prevalence (Gonzalez-Villoria and Valverde-Garduno 2016). The reports above showed that most countries have bacteria resistance diversity to some antibiotics. The bacteria resistance probably results in difficulties and expensive treatments. MDR bacteria which cannot be handled properly perhaps will cause a high mortality and morbidity rate. One of the solutions that can be offered is to search and to find novel antibacterial compounds. Unfortunately, at the moment, the findings related to novel antibacterial compounds are still rare. New discoveries of antibacterial drugs have been slowing since over decades ago (Projan and Shlaes 2016). Due to the risk and novel antibacterial necessity, research on antibacterial compound from our environment is therefore needed.

Some bacteria can be used as a source of antibacterial compounds, which are Actinomycetes. Actinomycetes can be found in soil, but mostly in plant decomposition areas (Wang et al. 2016). The majority (66%) of rhizosphere actinomycetes were *Streptomyces* genera. This bacteria group has high guanine and cytocin contents (more than 51%) in their DNA which support a good metabolism for producing several bioactive compounds (Ventura et al. 2007). They are special microorganisms because of their ability to produce antibacterial compound in variance and abundance. That was evidenced by the discovery of 160 antibiotics for human therapy; 140 of them produced by actinomycetes (Berdy 2005). The inhibition effectivity of actinomycetes inhibiting marine, Antartic, and plant endophyte were different. Actinomycetes isolated from Antartic soil produce antimicrobial compounds against *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Lee et al. 2012). Marine actinomycetes *Streptomyces atratus* SCSIO ZH16Ma produce L-3-nitrotyrosine and L-2-amino-4-hexenoic acid as antimycobacterial in tuberculosis patient (Ma et al. 2017). The crude extract of endophytic actinomycetes of *Caesalpinia pyramidalis* inhibited *Candida albicans*, *Bacillus subtilis*, *Fusarium moniliforme*, and *Staphylococcus aureus* in various concentration. Their crude extract includes monoterpene, sesquiterpenes,

proanthocyanidin, triterpenes, and steroids group as antimicrobial. The species of endophytic actinomycetes which produces antimicrobial compounds is known as *Streptomyces parvulus* (Silva-Lacerda et al. 2016). Based on the different ability in some locations, the inhibition effectivity of rhizosphere actinomycetes was suspected of being different. In addition, although they were isolated from the same soil sample, the characters of bioactive compounds from the condition of the areas are different. The quality of bioactive compound produced by dry soil actinomycetes is better than that produced in wet area such as: more stable, good soluble, and bioavailable (Mohammadipanah and Wink 2016). It means that this bacteria group is still potential to be used as a source of novel antibacterial compound for some infectious diseases. Christine et al. (2018) reported that *Escherichia coli*, *Acinetobacter calcoaceticus*, *Citrobacter braakii*, and *Klebsiella pneumoniae* were found in UTI urine patients. In accordance with the requirement of new antibacterial compounds and the existence of actinomycetes in rhizosphere, this research conducted some methods to find novel antibacterial compounds from actinomycetes for bacteria from UTI patients as a target.

MATERIALS AND METHODS

Enrichment of actinomycetes and UTI's bacteria

This study started with the enrichment of two bacteria groups used in this study. The first group consisted of 21 actinomycetes from maize of rhizosphere soil in Nusa Tenggara Timur, Indonesia that was collected in Laboratory of Microbiology, IPB and routinely cultured on International Streptomyces Project (ISP) 4 (inorganic salts-starch agar) medium. Another group included was six UTI's bacteria (*Escherichia coli 1 (α-hemolysin)*, *E. coli 2 (α-hemolysin)*, *E.coli 3 (β-hemolysin)*, *Acinetobacter calcoaceticus*, *Citrobacter braakii*, and *Klebsiella pneumoniae*) routinely cultured on Eosin Methylene Blue Agar (EMBA) and Nutrient Agar (NA) medium. Actinomycetes were cultured at room temperature (27-30 ^{o}C) for 7-14 days, while UTI's bacteria were cultured at 37 ^{o}C for 24-48 hours.

Afterward, the screening step was conducted based on Kirby Bauer method (Bauer et al. 1966). The UTI's bacteria with concentration of 10^6 cfu/mL in NA medium was poured to Petri disc. Then, twelve-day old actinomycetes were cut with sterile straw of 6 mm at diameter and placed on NA medium containing the test bacteria. They were incubated at 37 ^{o}C for 24 hours. After that, inhibition area was measured and the inhibition index was calculated using the Inhibition Index (II) formula below. Isolates with the largest inhibition zone were selected to be cultured in liquid ISP 4 (inorganic salts-starch) medium and shaken on the shaker incubator at 120 x g speed, 27-30 ^{o}C for 12 days. The supernatant and pellet were divided using centrifuge (HERMILE Labortechnik GmbH type Z326 K) at speed of 4000 x g, 4 ^{o}C for 15 minutes. Supernatant was dropped at paper disc that was placed on NA medium with 10^6 cfu/mL concentration of UTI's bacteria. It was incubated at 37 ^{o}C for 24 hours. Then, the inhibition area was measured to confirm the antibacterial activity from bioactive compounds inside (Sharma et al. 2011).

$$\text{Inhibition index (II)} = \frac{\text{Diameter inhibition area (mm)} - \text{diameter colony (mm)}}{\text{Diameter colony (mm)}}$$

Characterization of bioactive compounds as antibacterial

For the characterization of antibacterial compounds from potential actinomycetes, the supernatant was extracted using a solvent. The supernatant was mixed with ethyl acetate with a ratio of 1:1 v/v. The solution was shaken gently for 30 minutes until it exhibited the liquid phase on top of the solution. Then, the top phase was evaporated at 50 ^{o}C for 30 minutes using a rotary evaporator. Afterward, the crude extract was dissolved in DMSO 100% until the solution reached 0.01 g/mL concentration. The solution was tested at paper disc placed on NA medium with 10^6 cfu/mL concentration test bacteria, then incubated at 37 ^{o}C for 24 hours. A commercial antibiotic was used as positive control (ciprofloxacin 500 mg) in 5 µg/mL concentration. After that, the crude extract was characterized using GC-MS in Center Laboratory of Forensic, Mabes Polri, South Jakarta, Indonesia. The equipment was set by oven started with temperature of 80 ^{o}C until 350 ^{o}C and the injector started at 300 ^{o}C with solvent separation 10:1. Then, the 1 µL sample volume was injected. Helium was used as a gas carrier at 0.9 mL/minutes speed. The operated column type was Agilent 19091S-433 (length 30 m, diameter 0.25 mm, thickness 0.25 µm) with 30 minute time scanning. The result of chromatogram was then matched with Willey (W10N14) library database to identify bioactive compounds inside.

Molecular identification of potential actinomycetes based on 16S rRNA gene and construction of phylogenetic tree

DNA of 12 day old potential actinomycetes was extracted using PrestoTM Mini gDNA Bacteria Kit. The total DNA concentration was quantified by using nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Afterward, DNA was amplified using PCR TI-Thermocycler (Biometra, Goettingen, Germany) to confirm bacterial identity. Amplification was carried out using primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16Sact1114R (5'-GAGTTGACCCCGGCRGT-3') (Martina et al. 2008). PCR solution was mixed approximately up to 50 µL of final concentration with the following composition of each reaction: 25 µL MyTaqTM Extract-PCR Kit, 4 µL of 25 pmol primer 27F, 4 µL of 25 pmol 16Sact1114R, 4 µL of 100 ng/µL DNA template, and 13 µL nuclease-free water. The PCR conditions were operated in pre-denaturation at 94 ^{o}C for 3 minutes and 30 cycles divided into two periods. The first period consisted of the following 15 cycles: denaturation at 94 ^{o}C for 3 minutes, annealing at 53 ^{o}C for 45 seconds, elongation for 1 minute at 72 ^{o}C. Then, the second period consisted of 15

cycles with the following steps: denaturation at 94 °C for 1 minute, annealing for 45 seconds at 55 °C, and elongation at 72 °C for 1 minute. After that, the 16S rRNA gene was sequenced by commercial company (First base). The sequencing result was trimmed using Seqtrace 0.90. Then, nucleotide base was aligned using Basic Local Alignment Sequence for nucleotide (BLAST-N). The phylogeny tree was constructed using Mega 6.0 by maximum likelihood method with bootstrap replication 1000x (Tamura et al. 2013).

RESULTS AND DISCUSSION

Screening of actinomycetes producing antibacterial compounds

Twenty-one actinomycetes, which were screened, showed 20 isolates inhibited at least one UTI's bacteria. There was variance at inhibition zone produced by actinomycetes. The spectrum of inhibition was narrow through the wide spectrum (Table 1). Isolate code ARJ 16, 24, and 36 exhibited wide spectrum and the largest inhibition zone than others.

Crude extract inhibition of potential actinomycetes and its bioactive compounds

The amount of extraction product varied at each isolate code. The crude extracts produced by ARJ 16, ARJ 24, and ARJ 36 were about 0.133 g, 0.287 g, 0.025 g, respectively. Supernatant and crude extract of selected actinomycetes inhibited all UTI's bacteria (Table 2). On the other hand, there were several bioactive compounds in ethyl acetate crude extract in various abundance. The highest abundance of bioactive compounds from ARJ 16, 24, and 36 were Propane, 1,2-dichloro, n-Hexadecanoic acid, and Carbonochloridic acid, 2-chloroethyl ester, respectively (Table 3).

Molecular identification of potential actinomycetes based on 16S rRNA gene

The 16S rRNA genes were successfully amplified by primer 27F and 16Sact1114R (~1100 bp). The result from NCBI BLAST showed that ARJ 16 and ARJ 24 were 99% similar with *Streptomyces* sp. strain TES 17, while ARJ 36 was 98% similar with *Streptomyces tendae* strain CMU RKDM 16 (Table 4). Based on the BLAST-N result, the phylogeny tree was constructed. It showed that ARJ 16 and 24 were closely related to *Streptomyces* sp. with bootstrap value 99, while ARJ 36 was closely related to *Streptomyces tendae* with bootstrap value 90 (Figure 1).

Each sequence of 16S rRNA gene potential actinomycetes was deposited to GenBank. Then, it obtained the accession number for ARJ 16, 24, and 36 were MK024250, MK024254, and MH633728, respectively.

Table 1. Inhibitory zone of rhizosphere actinomycetes against UTI's bacteria

Isolate code	UTI's bacteria											
	Escherichia Coli 1	II	*Escherichia coli* 2	II	*Acinetobacter calcoaceticus*	II	*Escherichia coli* 3	II	*Citrobacter braakii*	II	*Klebsiella pneumoniae*	II
ARJ 11	+	0.33	+	0.33	+	0.17	+	0.33	+	0.33	+	0.33
ARJ 15	+	0.5	+	0.33	+	0.33	+	0.67	-	-	+	0.5
ARJ 16	++	1.83	+++	3.17	+	0.33	+	0.4	+	0.17	+	0.33
ARJ 17	+	0.25	-	-	-	-	+	0.33	+	0.33	+	0.5
ARJ 21	-	-	+	0.42	+	0.58	+	0.5	+	0.17	+	
ARJ 23	+	0.17	+	0.25	+	0.25	+	0.42	+	0.33	+	0.33
ARJ 24	+	0.33	+	0.33	+	0.33	+	0.33	-	-	+++	3.5
ARJ 27	+	0.17	+	0.17	-	-	+	0.17	-	-	-	-
ARJ 28	+	0.33	+	0.25	+	0.33	+	0.5	+	1	+	0.33
ARJ 31	+	0.33	+	0.42	+	0.58	+	0.33	+	0.17	+	0.5
ARJ 32	+	0.67	+	0.5	+	0.33	+	0.83	+	0.67	+	0.83
ARJ 33	-	-	+	0.67	+	1.33	+	0.33	+	0.33	+	0.5
ARJ 36	+++	3.33	+	1.5	++	2.33	+	1.67	+++	3.25	++	1.83
ARJ 37	-	-	-	-	+	0.17	-	-	-	-	-	-
ARJ 38	+	0.33	-	-	+	0.5	+	0.67	+	0.67	+	0.67
ARJ 42	+	0.5	+	0.33	+	0.42	+	0.5	+	1.5	+	0.17
ARJ 43	+	0.17	+	0.17	+	0.5	-	-	+	0.25	+	0.58
ARJ 44	+	1.25	+	0.33	+	0.42	+	0.17	+	0.33	+	0.33
ARJ 47	-	-	-	-	-	-	-	-	-	-	-	-
ARJ 49	+	0.3	+	0.5	-	-	+	0.17	+	0.67	+	0.5
ARJ 51	+	1.25	+	0.33	+	0.42	+	0.17	+	0.33	+	0.33

Note: Inhibition Index (II): 0 (-), 0.1-1 (+), 1.1-3 (++), >3 (+++).

Table 2. Inhibition index of supernatant and crude extract to UTI's bacteria

		Inhibition index					
	Isolate code	***Escherichia coli* 1**	***Escherichia coli* 2**	***Acinetobacter calcoaceticus***	***Escherichia coli* 3**	***Citrobacter braakii***	***Klebsiella pneumoniae***
Supernatant	ARJ 16	1	0.67	4	0.83	0.83	0.67
	ARJ 24	0.83	0.33	3.67	0.83	0.83	0.67
	ARJ 36	1.5	1.17	4.33	0.83	0.83	0.83
Crude extract (0.01 g/mL)	ARJ 16	1	0.83	0.17	0.67	0.67	0.5
	ARJ 24	0.5	0.83	0.17	0.5	0.83	0.5
	ARJ 36	2.33	0.83	0.17	0.83	0.83	0.67
Ciprofloxacin (5 µg/mL)		2.17	2.17	0.17	6	NI	NI
DMSO (100%)		NI	NI	NI	NI	NI	NI

Note: NI: no inhibition

Table 3. Ten tops abundance of bioactive compounds in ARJ 16, 24, and 36 crude extract

Isolate code	Peak area (%)	Retention time (minutes)	Name of compounds	Formula	Molecular weight	Therapeutic activity	References
ARJ 16	16.92	4.554	Propane, 1,2-dichloro	$C_3H_6Cl_2$	112	Unknown	-
	12.36	4.172	Silane, chloromethylphenyl	C_7H_9ClSi	156	Unknown	-
	8.65	4.715	2-Chloroethyl carbonate	$C_3H_6Cl_2$	186	Unknown	-
	0.05	14.757	Tris (2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_4P$	662	Antioxidant	Vinuchakkaravarthy et al. (2010)
	0.01	4.826	Benzenediazonium, 4-hydroxy, hydroxide, inner salt	$C_6H_4N_2O$	120	Antibacterial	Johnson et al. (2013)
	0.01	4.962	1-Ethanol, 2- (ethylsulfinyl)	$C_4H_{10}O_2S$	122	Unknown	-
	0.01	5.200	2-Chloroethyl methyl sulfoxide	C_3H_7ClOS	126	Unknown	-
	0.01	5.319	Cyclobutanol	C_4H_8O	72	Antibacterial	Ara et al. (2012)
	0.01	10.900	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antibacterial	Pu et al. (2010)
	0.01	15.929	Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester	$C_{20}H_{22}N_2O_4$	354	Antifungal	Husein et al. (2016)
ARJ 24	9.23	10.934	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antibacterial	Pu et al. (2010)
	8.24	15.768	Phthalic acid, bis (7-methyl octyl) ester	$C_{26}H_{42}O_4$	418	Antibacterial	Ramakrishnan and Venkataraman (2011)
	6.65	15.938	Phthalic acid, 3-fluorophenyl hexadecyl ester	$C_{30}H_{41}FO_4$	484	Unknown	-
	6.53	15.428	Phthalic acid, isohexyl pentadecyl ester	$C_{29}H_{48}O_4$	460	Unknown	-
	5.59	16.108	Phthalic acid, 4-chloro-2-methyl phenyl tetradecyl ester	$C_{29}H_{39}ClO_4$	486	Unknown	-
	5.29	15.581	Phthalic acid, 4-methyl pent-2-yl nonyl ester	$C_{23}H_{36}O_4$	376	Unknown	-
	4.25	15.649	1,2-Benzenedicarboxylic acid, dinonyl ester	$C_{26}H_{42}O_4$	418	Antineuro-degenerative	Choi et al. (2009)
	3.57	12.175	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Antibacterial	Roy et al. (2010), Njume et al (2011)
	3.46	15.997	Phthalic acid, 3-fluorophenyl pentadecyl ester	$C_{29}H_{39}OFO_4$	470	Unknown	-
	2.24	11.189	Procucumenol	$C_{15}H_{22}O_2$	234	Anti-inflamation	Jang et al. (2004)
ARJ 36	23.19	3.706	Carbonochloridic acid, 2-chloroethyl ester	$C_3H_4Cl_2O_2$	142	Unknown	-
	1.90	5.048	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	93	Antibacterial	Kumar et al. (2010)
ARJ 36	1.45	10.935	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antibacterial	Pu et al. (2010)
	1.12	5.549	2-Furaldehyde, 5- (hydroxymethyl)	C6H6O3	126	Antioxidant, antifungal	Khan et al. (2012), Subramenium et al. (2018)
	0.61	5.685	Acetic acid, phenyl	$C_8H_8O_2$	136	Antibacterial	Thorp et al. (1998)
	0.53	3.952	1,3-Cyclopentanedione	$C_5H_6O_2$	98	Antibacterial	Hamza et al. (2015)
	0.53	4.139	4-Methoxy-5-ethyl imidazole	$C_6H_{10}N_{20}$	126	Unknown	-
	0.50	4.470	2-Furancarboxylic acid	$C_5H_40_3$	112	Antibacterial	Kuo et al. (2014)
	0.49	4.836	4H-Pyran-4-one, 2-hydroxy-3-methyl	$C_6H_60_3$	126	Unknown	-
	0.48	5.303	Furazan-3-carboxamide acid hydrazide, 4-amino	$C_3H_6N_6O$	142	Unknown	-

Table 4. BLAST result of actinomycetes 16S rRNA gene

Isolate code	Description	Query cover	E-value	Identity	Accession number
ARJ 16	*Streptomyces* sp. strain TES 17	100%	0.0	99%	KY511722
ARJ 24	*Streptomyces* sp. strain TES 17	100%	0.0	99%	KY511722
ARJ 36	*Streptomyces tendae* strain CMU-RKDM 16	100%	0.0	98%	LC310913

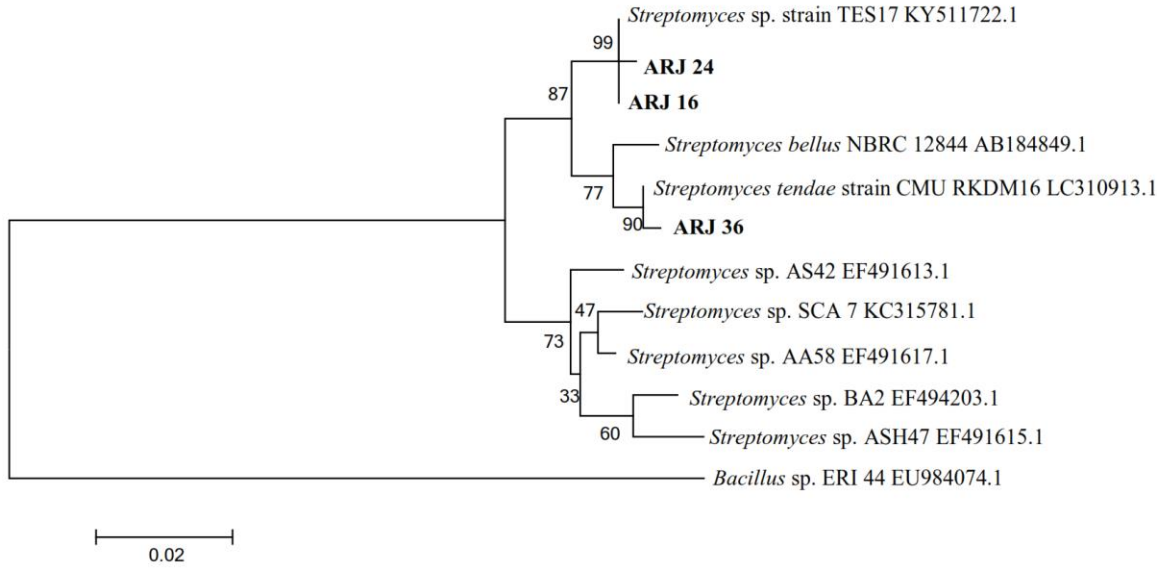

Figure 1. Phylogenetic tree of actinomycetes producing UTI's antibacterial using maximum likelihood method with bootstrap 1000 replicates. *Bacillus* sp. was used as out-group. Bar 0.02 indicated nucleotide substitution per site

Discussion

We have screened 21 actinomycetes against UTI's bacteria. They showed that 20 (95%) isolates inhibited at least one of UTI's bacteria group. Previous research reported, that actinomycetes from arid zone of Badia region, Jordan showed 30% inhibited the test bacteria (Saadoun and Gharaibeh 2003). Other cases showed that fifty isolates from farming soils in Turkey revealed antibacterial activity of 17 tested (39%) (Oskay et al. 2004). Then, all isolates from soil at Cukuva University, Turkey was also reported to be inhibited test bacteria in medium glycerol-yeast agar, at least one test bacteria (Sarigullu et al. 2013). It gave evidence that actinomycetes were able to produce many antibacterial compounds.

In this study, isolate code of ARJ 16, 24, and 36 were mentioned as potential actinomycetes because they showed greatest inhibition zone in terms of cell activity. That becomes a reason to check the supernatant and crude extract inhibition of each potential isolates for the next test. The supernatant and crude extract inhibited UTI's bacteria. This fact indicated that antibacterial compounds were excreted to liquid medium by the cell. However, the supernatant ability of each isolate was different. Several supernatants showed smaller inhibition zone than cell activity. This fact is due to the character of antibacterial compounds and sensitivity level of test bacteria. Previous research has shown that bioactive compounds are more stable in immobilized cell than free cell (Dhananjeyan et al. 2010). The similar condition happened to crude extract. It could inhibit UTI's bacteria that indicated antibacterial compound was successfully extracted by ethyl acetate. This case can be explained by various bioactive compounds revealed by GC-MS (Table 3).

Based on the above data, ARJ 16 and 24 crude extract inhibition index to all *E. coli* strain showed smaller than ciprofloxacin. The same thing happened to ARJ 36 crude extract to *E. coli* strain 2 and 3. This fact is due to the variance of bioactive compounds included in ARJ 16, 24, and 36 crude extract that gave another therapeutic activity besides antibacterial, such as antioxidant, antiinflammation, antifungal, or antineuro-degenerative (Table 3). Meanwhile, ciprofloxacin was antibiotic with pure antibacterial compound. So, in this case, ciprofloxacin pointed out the greater action than crude extract. However, the data indicated that all crude extract inhibition index was the same with ciprofloxacin to *A. calcoaceticus*. It was suspected that bacteria sensitivity by antibiotic was decreased. Other things were exactly different by all of crude extracts against *C. braakii* and *K. pneumoniae*. They were not inhibited by ciprofloxacin and showed large inhibition index by crude extract. It showed that they had ciprofloxacin resistance. In addition, Reis et al. (2016) also reported that *K. pneumoniae* from UTI's patient showed resistant to ciprofloxacin and many other antibiotics.

Each bioactive compounds had different abundance and retention time. The ARJ 16 crude extract had Propane, 1,2-dichloro as bioactive compound with highest abundance included. Thirty percent of ARJ 16 crude extract was reported as antibacterial. Benzenediazonium, 4-hydroxy-, hydroxide, inner salt was one of bioactive compound in ARJ 16 crude extract that reported as antibacterial against *E.coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* (Johnson et al. 2013). Then, the ARJ 24 ethyl acetate crude extract revealed 30% antibacterial activity. Interestingly, in this extract, there was procucumenol as anti-inflammation (Table 3). It could be a benefit in the future because, in the same extract, we got combination between antibacterial and anti-inflammation those their work always complemented each other. One of antibacterial compounds which becomes the property compound of ARJ 16, 24, and 36 crude extract was hexadecanoic acid. It was fatty acids group and reported to inhibit *E.coli* and *Salmonella* sp. growth (Pu et al. 2010). The ARJ 36 crude extract showed that most of them (50%) are antibacterial compounds. Carbonochloridic acid, 2-chloroethyl ester was highest abundance included. Several antibacterial compounds in ARJ 36 was 1,3-Cyclopentanedione and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl. That was alicyclic hydrocarbon and flavonoid, respectively which inhibit *K. pneumoniae*, *E. coli*, and *P. aeruginosa* (Hamza et al. 2015). Acetic acid, phenyl was carboxylic acid group that was reported as ear dunk human antibacterial (Thorp et al. 1998).

Three potential actinomycetes were identified as 16S rRNA genes. Based on BLAST result, they were similar to the genus *Streptomyces*. This genus is known as a huge antibacterial producer. As previous study reported, *Streptomyces* sp. collected from soil sample of agriculture showed antibacterial activity against *Staphylococcus epidermidis* (Kumar et al. 2014). The same species from hills soil produced antibacterial compounds for *Enterobacter aerogenes* (Balachandran et al. 2015). In addition, the genus *Streptomyces* also inhibit methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), and extended-spectrum lactamase (ESBL) producing bacteria (Sharma et al. 2011). In this research, although ARJ 16 and 24 were the same species, we suspected that they were different strain. It is caused by the different bioactive compound which they produced. It could be proposed that *Streptomyces* sp strain ARJ 16, *Streptomyces* sp. strain ARJ 24, and *Streptomyces tendae* could inhibit UTI's bacteria growth by producing many bioactive compounds, especially antibacterial compounds. This study could be a reference for the development of UTI's antibacterial agents in the future. Perhaps, we could improve the antibacterial property by enhancing the culture condition, extraction method, or molecular method.

REFERENCES

Ara I, Bukhari NA, Solaian D, Bakir MA. 2012. Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis. J Med Plants Res. 6 (45): 5688-5694.

Balachandran C, Duraipandiyan V, Emi N, Ignacimuthu S. 2015. Antimicrobial and cytotoxic properties of *Streptomyces* sp. (ERINLG-51) isolated from Southern Western Ghats. South Indian J Biol Sci. 1 (1): 7-14.

Bauer AW, Kirby WMM, Sherris JC, dan Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 36: 493-496.

Berdy J. 2005. Bioactive microbial metabolites, a personal view. J Antibiotic. 58 (1): 1-26.

Choi SJ, Kim MJ, Heo HJ, Kim JK, Jun WJ, Kim HK, Kim E, Kim MO, Cho HY, Hwang H, Kim YJ, Shin D. 2009. Ameliorative effect of 1,2-benzene dicarboxylic acid dinonyl ester against amyloid β peptide-induced neurotoxicity. Amyloid 16 (1): 15-24.

Christine G, Budiarti S, Astuti RI. 2018. Diversity of urinary tract infection bacteria in children in Indonesia based on metagenomic approach. Biodiversitas 19 (4): 1375-1381.

Dhananjeyan V, Selvan N, Dhanapal K. 2010. Isolation, characterization, screening, and antibiotic sensitivity of actinomycetes from locally (near MCAS) collected soil samples. J Biol Sci. 10 (6): 514-519.

ECDC (European Centre for Disease Prevention and Control). 2015. Antimicrobial resistance surveillance in Europe 2014. European Centre for Disease Prevention and Control, Stockholm.

Gonzalez-Villoria AM, Valverde-Garduno V. 2016. Antibiotic-resistant *Acinetobacter baumannii* increasing success remains a challenge as a nosocomial pathogen. J Pathog. : 1-11.

Hamza LF, Sabreen A, Kamal, Hameed IH. 2015. Determination of metabolites products by *Penicillium expansum* and evaluating antimicrobial activity. J Pharmacognosy Phytother.7 (9): 194-220.

Hussein HM, Hameed IH, Ibraheem OA. 2016. Antimicrobial Activity and Spectral Chemical Analysis of Methanolic Leaves Extract of *Adiantum Capillus-Veneris* Using GC-MS and FT-IR Spectroscopy. Inter J Pharmacogn Phytochem Res. 8 (3): 369-385.

Jang MK, Lee HJ, Kim JS, Ryu J. 2004. A curcuminoid and two sesquiterpenoids from *Curcuma zedoaria* as inhibitors of nitric oxide synthesis in activated macrophages. Arch Pharm Res. 27 (12): 1220-1225.

Johnson OO, Ayoola GA, Adenipekun T. 2013. Antimicrobial Activity and the Chemical Composition of the Volatile Oil Blend from *Allium sativum* (Garlic Clove) and *Citrus reticulata* (Tangerine Fruit). Int J Pharm Sci Drug Res. 5 (4): 187-193.

Khan H, Saeed M, Khan MA, Khan I, Ahmad M, Muhammad N, Khan A. 2012. Antimalarial and free radical scavenging activities of rhizomes of *Polygonatum verticillatum* supported by isolated metabolites. Med Chem Rest. 21: 1278-1282.

Kumar PP, Kumravel S, Lalitha C. 2010. Screening of antioxidant, total phenolics, and GC-MS study of *Vitex negundo*. Afr J Biochem Res. 4 (7): 191-195

Kumar PS, Duraipndiyan V, Ignacimuthu S. 2014. Isolation, screening and partial purification of antimicrobial antibiotics from soil *Streptomyces* sp. SCA 7. Kaohsiung J Med Sci. 30: 435-446.

Kuo P, Chen G, Yang M, Lin Y, Peng C. 2014. Chemical Constituents from the Fruits of *Forsythia suspensa* and their antimicrobial activity. BioMed Res Int.: 1-7.

Lee LH, Cheah YK, Sidik SM, Mutalib NA, Tang YL, Lin HP, Hong K. 2012. Molecular characterization of Antarctic actinobacteria and screening for antimicrobial metabolite production. World J Microbiol Biotechnol. 28: 2125-2137.

Ma J, Huang H, Xie Y, Liu Z, Zhao J, Zhang C, Jia Y, Zhang Y, Zhang H, Zhag T, Ju J. 2017. Biosynthesis of ilamycins featuring unusual building blocks and engineered production of enhanced anti-tuberculosis agents. Nat Commun 18 (391): 1-10.

Martina K, Jaan K, Tamas F, Ladislav C, Marek O, Genevieve GL, Yvan ML, Marketa S. 2008. Development of a 16S rRNA gene-based prototype microarray for the detection of selected actinomycetes genera. Anton Leeuw Int J G. 94: 439-453.

Mohammadipanah F, Wink J. 2016. Actinobacteria from arid and desert habitats: diversity and biological activity. Front Microbiol. 6 (1541): 1-10.

Navon-Venezia S, Kondratyeva K, Carattoli A. 2017. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 41: 252-275.

Njume C, Afolayan AJ, Green E, Ndip RN. 2011. Volatile compounds in the stem bark of *Sclerocarya birrea* (Anacardiaceae) possess

antimicrobial activity against drug-resistant strains of *Helicobacter pylori*. Int J Antimicrob Agents 38: 319-324.

Oskay M, Tamer O, Azeri C. 2004. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr J Biotechnol. 3 (9): 441-446.

Parajuli NP, Maharjan P, Parajuli H, Joshi G, Paudel D, Sayami S, Khanal PR. 2017. High rates of multidrug resistance among uropathogenic *Escherichia coli* in children and analyses of ESBL producers from Nepal. Antimicrob Resist Infect Control 6 (9): 1-7.

Projan SJ, Shlaes DM. 2004. Antibacterial drug discovery: is it all downhill from here?. Clin Microbiol Infect 10 (Suppl. 4): 18-22.

Pu ZH, Zhang YQ, Yin ZQ, Xu J, Jia RY, Lu Y, Yang F (2010) Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3, 4-diyl ester from neem oil. Agric Sci China 9:1236-1240.

Ramakrishnan S, Venkataraman R. 2011. Screening of antioxidant activity, total phenolics and gas chromatography-mass spectrophotometer (GC-MS) study of ethanolic extract of *Aporosa lindleyana* Baill. Afr J Biochem Res 5 (14): 360-364.

Reis ACC, Santos SRS, Souza SC, Saldanha MG, Pitanga TN, Oliveira RR. 2016. Ciprofloxacin resistance pattern among bacteria isolated from patients with community-acquired urinary tract infection. Rev Inst Med Trop. Sao Paulo: 53-58.

Roy S, Rao K, Bhuvaneswari C, Giri A, Mangamoori LN. 2010. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World J Microbiol Biotechnol. 26: 85-91.

Saadoun I, Gharaibeh R. 2003. The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J Arid Environ. 53: 365-371.

Sarigullu FE, Karadeniz E, Untac I, Colak O. 2013. Determination of antibacterial activities of isolated *Streptomyces* strains from soil at Çukurova University in Turkey. J Food Agric Environ. 11 (2): 922-924.

Sharma D, Kaur T, Chadha BS, Manhas RJ. 2011. Antimicrobial activity of actinomycetes against multidrug-resistant *Staphylococcus aureus*, *E. coli*, and various other pathogens. Trop J Pharm Res 10 (6): 801-808.

Silva-Lacerda GR, Santana RCF, Vicalvi-Costa MCV, Solidonio EG, Sena KXFR, Lima GMS, Araujo JM. 2016. Antimicrobial potential of actinobacteria isolated from the rhizosphere of the Caatinga biome plant *Caesalpinia pyramidalis* Tul. Genet Mol Res. 15 (1): 1-12.

Subramenium GA, Swetha TK, Iyer PM, Balamurugan K, Pandian SK. 2018. 5-hydroxymethyl-2-furaldehyde from marine bacterium *Bacillus subtilis* inhibits biofilm and virulence of *Candida albican*. Microbiol Res. 207: 19-32.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. Mega 6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30: 2725-2729.

Teerawattanapong N, Panich P, Kulpokin D, Ranong SN, Kongpakwattana K, Saksinanon A, Goh B, Lee L, Apisarnthanarak A, Chaiyakunapruk N. 2018. A systematic review of the burden of multidrug-resistant healthcare-associated infections among intensive care unit patients in Southeast Asia: the rise of multidrug-resistant *Acinetobacter baumannii*. Infect Control Hosp Epidemiol.; 1-9.

Thorp MA, Kruger J, Oliver SS, Nilssen ELK, Prescot CAJ. The antibacterial activity of acetic acid and Burow's solution as topical otological preparations. J Laring Ontolog. 112: 925-928.

Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF, Sinderen DV. 2007. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol Mol Biol Rev. 71 (3): 495-548.

Vinuchakkaravarthy T, Sangeetha CK, Velmurugan D. 2010. Tris (2,4-di-tert-butylphenyl) phosphate. Acta Cryst. 66: 2207-2208.

Wang C, Dong D, Wang H, Muller K, Qin Y, Wang H, Wu W. 2016. Metagenomic analysis of microbial consortia enriched from compost: new insights into the role of Actinobacteria in lignocellulose decomposition. Biotechnol Biofuels 9 (22): 1-17.

WHO. 2018. Antimicrobial resistance. World Health Organization, Geneva https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.