Xylan-degrading ability of thermophilic *Actinobacteria* isolated from soil in a geothermal area

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Manuscript received: 15 October 2019. Revision accepted: 14 December 2019.

Abstract. *Rachmania MK, Ningsih F, Setyaningsih PP, Syafitri WA, Sari DCAF, Yabe S, Yokota A, Oetari A, Sjamsuridjal W. 2020. Xylan-degrading ability of thermophilic* Actinobacteria *isolated from soil in a geothermal area. Biodiversitas 21: 144-154.* This study was conducted to obtain the potential isolates of xylan-degrading thermophilic *Actinobacteria*. Seventeen isolates were obtained from the soil samples collected from Cisolok geysers in West Java, Indonesia. These isolates were screened for their xylan-degrading ability on minimal medium with the addition of 0.5% xylan as substrate, and were incubated at various temperatures for 7 days. A total of 15, 14, 4, and 3 isolates showed a xylan-degrading ability at 45°C, 50°C, 55°C, and 60°C, respectively. The three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) that showed xylan-degrading ability at 60°C on 0.5% xylan were further tested by using minimal medium with the addition of 0.1% RBB-xylan as a substrate. The results showed that these isolates were also able to utilized RBB-xylan at 45 to 60°C. The xylanolytic activity of the crude enzymes from the three isolates on minimal medium containing 0.5% xylan or 0.1% RBB-xylan as substrates, indicated that these isolates were able to produce extracellular xylanase. This study revealed that the soil of Cisolok geysers served as an ideal source for finding the thermophilic *Actinobacteria* which produced xylanase at high temperatures.

Keywords: Cisolok geysers, soil, thermophilic *Actinobacteria,* xylanase

INTRODUCTION

Phylum *Actinobacteria* is one of the largest taxonomic groups within the domain *Bacteria*, and it constitutes Gram-positive bacteria with high GC DNA content (Ventura et al. 2007). Most of the members of this phylum are mycelium-forming bacteria (Barka et al. 2016). The majority of *Actinobacteria* are soil inhabitants, and they contribute to the decomposition process and nutrient cycling (Goodfellow and Williams 1983). *Actinobacteria* are known to be potential producers of various secondary metabolites, such as antibiotics, enzymes, herbicides, and antifungal (Barka et al. 2016).

Thermophilic *Actinobacteria* are adapted to high temperatures ranges between 40 to 80°C. Moreover, thermophilic *Actinobacteria* thrive in diverse environments, such as sea sediment, hydrothermal vents, desert, geothermal sites, and compost heaps. Their ability to survive under high temperatures is attributed to the stability of their cellular components, such as by production of specific enzymes (Shivlata and Satyanarayana 2015).

Thermophilic *Actinobacteria* play a major role in industrial fields due to their ability to produce various thermostable enzymes, such as amylase, cellulase, lipase, protease, and xylanase (Singh et al. 2013). In recent years, pulp and paper industries are faced an environmental problem due to their utilization of chlorinated organic substances which are toxic, persistent, and harmful to organisms and environments (Beg et al. 2001). Xylanases produced by microorganisms have gained great attention as an alternative substitution agent to toxic chorine chemicals used in pre-bleaching of kraft pulps process due to their environmentally friendly and economically cheap technologies (Walia et al. 2017). Consequently, the use of xylanases in several industrial processes has stimulated various researches that investigate the characteristics of xylanolytic enzymes (Beg et al. 2001).

Xylan is a major polysaccharide component of hemicellulose, which is found in lignocellulosic biomass of plant cell walls (Moreira and Filho 2016). Xylan consists of xylose units linked by β-1,4 glycosyl linkages (Singh et al. 2013). Complete degradation of xylan requires synergistic hydrolytic enzymes, including enzymes responsible for the degradation of xylan backbone and its various side chain groups (Kumar et al. 2018). Xylanases catalyze the hydrolysis of xylan by cleaving the β-1,4-xylosidic bonds in the backbone of xylan, which consists of xylose units (Kumar et al. 2018). Endo-xylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) are the major enzymes involved

in xylan degradation (Juturu and Wu 2012). Thus, the extracellular endo-1,4-β-xylanase produced by bacteria could be detected by the presence of a clear zone around colonies grown on xylan-containing medium after being stained with Congo red (MacKenzie and Williams 1984).

Xylanases have been utilized in various industrial applications, e.g. bleaching in the paper industry, liquefaction of coffee mucilage, maceration of vegetable, extraction of pigments and flavors, bioremediation of agricultural wastes (Kulkarni et al. 1999), and saccharification of xylan (Juturu and Wu 2012). Most of these industrial processes require stable xylanases at high temperatures (Kumar et al. 2018); for example, the conversion of xylan into xylose during saccharification process occurs at 40−80°C (Juturu and Wu 2012). Therefore, the findings of microorganisms that are able to produce stable and active xylanases at high temperatures will have a great impact on various industrial applications (Beg et al. 2001).

Production of thermostable xylanase has been reported in various genera of *Actinobacteria*, such as *Actinomadura*, *Cellulosimicrobium*, *Streptomyces*, and *Thermobifida* (Walia et al. 2017). *Actinomadura xylanilytica* is one of the members of the genus *Actinomadura* that can degrade xylan (Zucchi et al. 2013). However, thermophilic *Actinobacteria* are rarely explored due to the difficulties in isolation and maintenance. Consequently, thermophilic xylanase-producing *Actinobacteria* are still rarely reported (Singh et al. 2013).

Bacterial communities in geothermal areas in Indonesia are still rarely explored, although these communities are potential producers of thermostable enzymes. Cisolok geothermal area in West Java, Indonesia is one of potential habitat from where thermophilic bacteria can be isolated (Mawarid et al. 2016; Yokota et al. 2016; Ningsih et al. 2019). Yokota et al. (2016) have proposed a new species of thermophilic bacterium, namely *Paenibacillus cisolokensis*, isolated from litter of Cisolok geysers, which can hydrolyze various substrates, such as starch, xylan, and cellulose at temperature of 50°C. Another thermophilic bacterium, *Brevibacillus* sp. UICC B-76, isolated from litter of Cisolok geysers, demonstrated xylanase, amylase, and cellulase activities at temperature of 50°C (Mawarid et al. 2016). Thus, the Cisolok geothermal area is a promising source of thermophilic enzyme producers.

In our previous study (Syafitri et al. 2019), we reported the growth and starch-degrading abilities from 17 *Actinobacteria* isolates obtained from soil of Cisolok geysers, West Java, Indonesia. A total of 17, 16, 6, and 5 isolates were able to grow at temperatures of 45, 50, 55, and 60°C, respectively, and 15 isolates showed starch-degrading ability at 45°C. The results indicating that all of the investigated *Actinobacteria* isolates were thermophiles and showed amylolytic activity. Three potential isolates, designated SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4, showed amylolytic activity at 60°C and were identified based on 16S rRNA gene as *Actinomadura keratinilytica*. We also reported in a separate paper (Setyaningsih et al. 2019) the cellulose-degrading ability of those 17 *Actinobacteria* isolates, which showed that 15 out of 17 isolates were able

to degrade carboxymethyl cellulose (CMC) at 45°C. The results also showed that three potential isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) were able to degrade both CMC and microcrystalline cellulose (MCC) substrates at 60°C. However, the study of the xylan-degrading ability from these 17 thermophilic *Actinobacteria* isolates has not been performed yet.

Studies on thermophilic *Actinobacteria* community in geothermal areas in Indonesia not only can contribute to discovering the potential sources of thermostable enzymes but also expanding the knowledge on the diversity of thermophilic *Actinobacteria* in Indonesia. This study aimed to obtain the potential isolates of xylan-degrading thermophilic *Actinobacteria* isolated from soil of Cisolok geysers, a geothermal area in West Java, Indonesia.

MATERIALS AND METHODS

Microorganisms

Seventeen thermophilic *Actinobacteria* isolates and the type strain of *Actinomadura keratinilytica* NBRC 105837[T] were used in this study. Seventeen *Actinobacteria* isolates were obtained from 5 soil samples collected from 3 locations around Cisolok geysers, a geothermal area in West Java, Indonesia. The information on the soil samples, sampling sites, and isolates deposition in Culture Collection have been reported in our previous study (Syafitri et al. 2019). The type strain of *Actinomadura keratinilytica* NBRC 105837[T] was obtained from NITE Biological Resource Center (NBRC) Culture Collection, Japan.

Screening of xylan-degrading ability

The 17 thermophilic *Actinobacteria* isolates were screened for xylan-degrading ability using xylan as substrate according to Meddeb-Mouelhi et al. (2014). Actinobacteria isolates were grown on minimal (Mm) agar plates with addition of 0.5% (w/v) xylan. The Mm agar without addition of xylan was served as a control. The plates were incubated up to 7 days at temperatures of 45, 50, 55, and 60°C. Xylan hydrolysis was detected by flooding the plates with 0.2% (w/v) Congo red solution and destained by two times washing with 1 M NaCl solution. The presence of a clear zone around the colonies indicated a positive result for xylan-degrading ability. The experiment was carried out in triplicates.

The xylan-degrading ability of the potential thermophilic *Actinobacteria* isolates and the type strain *Actinomadura keratinilytica* NBRC 105837[T] were tested by using 0.5% (w/v) xylan and 0.1% (w/v) RBB-xylan (Birchwood xylan dyed by Remazol Brilliant Blue R, Azo-xylan, Megazyme) as substrates, according to the methods described by Meddeb-Mouelhi et al. (2014) and Jeffrey et al. (2011) respectively. The isolates were grown on Mm agar plates with the addition of 0.5% xylan and 0.1% RBB-xylan, incubated at temperatures of 45, 50, 55, and 60°C up to 7 and 3 days, respectively. The experiment was carried out in duplicates.

Examination of extracellular xylanase production was performed according to Yabe S 2019, pers. com. and

Ningsih F 2019, pers. com. The potential isolates and type strain were grown on modified Bennett's (Jones 1949) broth, with glucose replaced by glycerol, and incubated at 45°C for 7 days as pre-inoculated culture. Cell biomass was harvested by centrifugation at 8,000 rpm, 4°C for 10 minutes, and washed twice with sterile deionized water. Cell biomass was inoculated into Mm broth containing 0.5% xylan and incubated at 45°C for 7 days as main culture. Centrifugation was performed to separate cell biomass and culture supernatants containing crude enzyme. Extracellular xylanase production was examined by placing 50 µl of culture supernatants on the surface of Mm plates containing 0.5% xylan and 0.1% RBB-xylan, followed by incubation at various temperatures (45, 50, 55, and 60°C) for 24 hours. Congo red staining method was performed for detection of extracellular xylanase production on 0.5% xylan. The formation of clear zone indicated positive results for xylanolytic activity.

Phylogenetic analyses

The operational taxonomic units (OTUs) and their accession numbers of the 16S rRNA gene sequences in the international DNA database used in the phylogenetic analyses are presented in Table 1. Multiple alignments of the 16S rRNA gene sequences was carried out using MEGA 7.0.26 software (Kumar et al. 2016) and all gap positions were excluded. The phylogenetic tree was constructed using the neighbor-joining (NJ) (Saitou and Nei 1987), maximum likelihood (ML) (Felsenstein 1981), and minimum evolution (ME) (Rzhetsky and Nei 1992) methods. The Kimura two-parameter substitution model was used to obtain the evolutionary distances (Kimura 1980). The robustness value of individual branches of the phylogenetic tree was obtained using bootstrap analysis with 1,000 replications (Felsenstein 1985).

Table 1. The OTUs and their accession numbers of the 16S rRNA gene sequences used in the phylogenetic analyses.

Strain	Accession number
Actinomadura barringtoniae GKU 128[T]	KF667497
A. fibrosa ATCC 49459[T]	AF163114
A. fulvescens IFO 14347[T]	U49005
A. gamaensis NEAU-Gz5[T]	KT989505
A. geliboluensis A8036[T]	HQ157187
A. hallensis H647-1[T]	DQ076484
A. hibisca NBRC 15177[T]	BCRO01000158
A. keratinilytica WCC-2265[T]	EU637009
A. kijaniata NBRC 1422[T]	BCQR01000335
A. madurae DSM 43067[T]	X97889
A. miaoliensis BC 44T-5[T]	EF116925
A. montaniterrae CYP1-1B[T]	LC126428
A. namibiensis DSM 44197[T]	AJ420134
A. nitritigenes DSM 44137[T]	AY035999
A. oligospora ATCC 43269[T]	AF163118
A. rhizosphaerae SDA37[T]	LC369500
A. rubrobrunea NBRC 15275[T]	BCQU01000204
A. rudentiformis HMC1[T]	DQ285420
A. syzygii GKU 157[T]	KF667496
A. viridilutea IFO14480[T]	D86943
A. xylanilytica BK147[T]	FR692101
Streptosporangium album DSM 43023[T]	X89934

Observation of morphological properties of potential *Actinobacteria* isolates

The potential *Actinobacteria* isolates were grown on modified Bennett's medium (Jones 1949), for 14 days at temperature of 45°C. The modified Bennett's medium, with the addition of 2% agar (HiMedia) and 2% gellan gum (Wako) as solidifying agents, were used to observe substrate and aerial mycelia, respectively. The morphological properties of the potential *Actinobacteria* isolates were observed under a Hirox KH-8700 digital microscope (Hirox Co. LTD, Japan), and were compared with the species description provided in the Bergey's Manual of Systematic Bacteriology: The *Actinobacteria*, parts A and B (Goodfellow et al. 2012).

RESULTS AND DISCUSSION

Screening of xylan-degrading ability

In this study, 15, 14, 4, and 3 isolates showed xylan-degrading ability at temperatures of 45, 50, 55, and 60°C, respectively. The results for screening of xylan-degrading ability from 17 thermophilic *Actinobacteria* isolates grown on Mm medium with the addition of 0.5% soluble xylan as substrate are shown in Table 2. The formation of clear zones around the colonies grown on 0.5% xylan following Congo red staining is shown in Figure 1. The formation of clear zone indicated the xylan-degrading ability of the colonies. According to MacKenzie and Williams (1984), the formation of clear zones on the screening medium was due to the production of extracellular xylanase. The binding interaction between polysaccharides and Congo red forms a red coloration. Xylanases play a role in the binding reaction as indicated by the formation of a clear zone, due to the hydrolysis of xylan into its oligomeric or monomeric units as a result of the β-1,4-xylan linkages cleavage.

Table 2. Xylan-degrading ability of the thermophilic *Actinobacteria* isolates grown on minimal medium containing 0.5% xylan as substrate at temperatures of 45, 50, 55, and 60°C for 3 and 7 days of incubation.

No.	Isolate	45°C		50°C		55°C		60°C	
		3d	7d	3d	7d	3d	7d	3d	7d
1	SL1-1-R-2	+	+	+	+	*	*	n/a	n/a
2	SL1-1-R-4	+	+	+	+	*	*	n/a	n/a
3	SL1-1-R-7	+	+	+	+	*	*	n/a	n/a
4	SL1-1-R-8	+	+	+	+	*	*	n/a	n/a
5	SL1-2-R-2	+	+	+	+	+	+	+	+
6	SL1-2-R-3	+	+	+	+	+	+	+	+
7	SL1-2-R-4	+	+	+	+	+	+	+	+
8	SL2-2-R-1	+	+	+	+	+	+	*	*
9	SL2-2-R-12	+	+	+	+	*	*	n/a	n/a
10	SL2-2-R-15	-	-	-	-	-	-	-	-
11	SL3-1-R-14	+	+	+	+	*	*	*	*
12	SL3-1-R-16	-	-	-	-	-	-	-	-
13	SL3-2-R-5	+	+	+	+	*	*	n/a	n/a
14	SL3-2-R-17	+	+	+	+	*	*	n/a	n/a
15	SL3-2-R-18	+	+	*	*	*	*	n/a	n/a
16	SL3-2-R-33	+	+	+	+	*	*	n/a	n/a
17	SL3-2-R-37	+	+	+	+	*	*	n/a	n/a

Note: (+) indicates positive result for xylan-degrading ability; (-) indicates negative result for xylan-degrading ability; (*) indicates no growth; and (n/a) indicates that the experiment was not conducted.
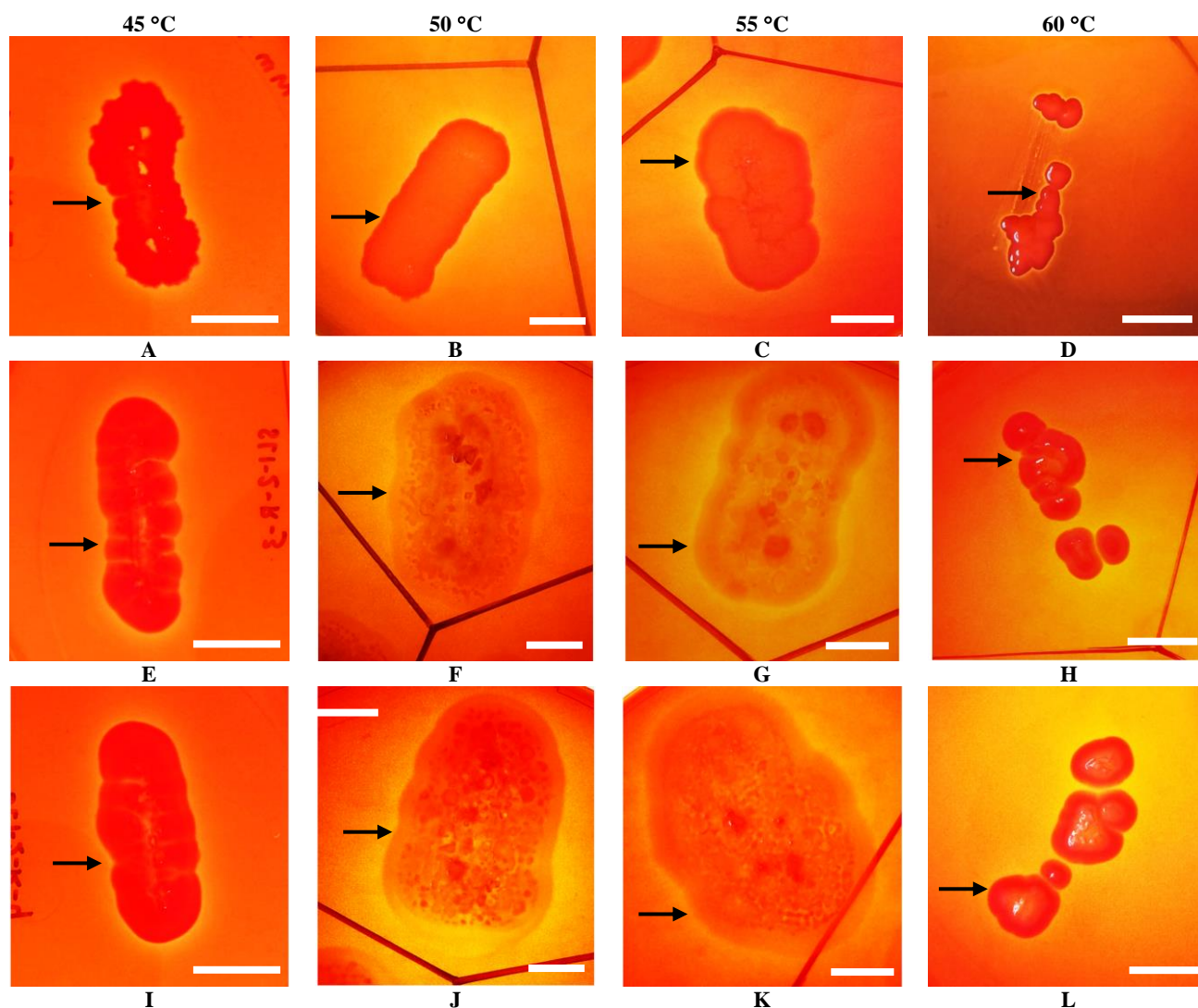
Figure 1. Clear zone formation in SL1-2-R-2 (a-d), SL1-2-R-3 (e-h), and SL1-2-R-4 (i-l) grown on minimal medium with addition of 0.5% xylan at temperatures of 45, 50, 55, and 60°C. Bar = 1 cm.

Among the 17 isolates, two isolates (SL2-2-R-15 and SL3-1-R-16) were able to grow on 0.5% xylan plates, but did not show xylan-degrading ability within 7 days of incubation at temperatures of 45, 50, 55, and 60°C (Table 1). These two isolates possibly could hydrolyze other organic components, such as agar and yeast extract in Mm medium as nutrients. As mentioned by Suzuki et al. (2003), agar is a polysaccharide consisting of agarose and agaropectin, and could be hydrolyzed by cleaving the β-1,4 linkage in agarose using β-agarase, producing neoagarooligosaccharides. Yeast extract is an essential organic carbon and nitrogen source for growth of microorganisms (Hakobyan et al. 2012), and it contains nucleic acid, lipid, protein, vitamins, and minerals (Chae et al. 2001).

According to Walia et al. (2017), xylanolytic activity is affected by physical parameters, such as temperature, pH, incubation period, and nutrients contents of the culture medium. The Mm medium used in this study contained metal ionic compounds, such as Mg^{2+} on $MgSO_4$ that may play a role as enzyme activator. Juturu and Wu (2012) mentioned that the metal ions, such as Mg^{2+} and Ca^{2+}, are enzyme activators for xylanase production.

Fifteen out of 17 (88%) thermophilic *Actinobacteria* isolates that isolated from soil of Cisolok geysers showed the xylan-degrading ability. This study suggests that thermophilic *Actinobacteria* isolates obtained from the soil samples of Cisolok geysers are potential producers of xylanase. They might be contributed to plant litter biomass degradation in Cisolok geysers or hot spring. Cisolok geothermal area is surrounded by vegetation of the forest with continuous litter fall which forms biomass in the geysers' area. The litter from the plant biomass might contain xylan polymers, and it might have been decomposed in the soil in Cisolok geothermal area. According to Moreira and Filho (2016), xylan is a major component of hemicellulose, which is found in plant cell walls. Thus, bacteria that possess xylanase could use it as

an organic nutrient. According to Lee et al. (2018), *Actinobacteria* was one of the classes that contributed to almost 69% of the total population in the degraded foliage microbiota from foliage-rich hot spring in Sungai Klah, Malaysia, based on metagenomic analysis. Our previous studies, Mawarid et al. (2016) and Yokota et al. (2016), have reported the xylan-degrading ability of thermophilic bacteria from the phylum *Firmicutes* isolated from litter in Cisolok geyser, e.g. *Brevibacillus* sp. UICC B-76 and *Paenibacillus cisolokensis* LC2-13A[T], respectively. The plant litter degrading ability of the thermophilic bacteria from the phylum *Firmicutes* also reported by Lee et al. (2018). They found that the dominant phyla in the microbiota of degraded foliage from the deeper part of hot spring were *Firmicutes* and *Proteobacteria*.

Phylogenetic tree analyses

The taxonomic affiliation of the three potential isolates obtained from this study (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) was clarified by constructing a phylogenetic tree based on the NJ, ME, and ML methods, using the Kimura two-parameter substitution model and the OTUs from their closely related species. The results of the phylogenetic analyses involving the three potential thermophilic

Actinobacteria isolates are shown in Figure 2. The three *Actinobacteria* isolates formed a monophyletic cluster with the type strain of their most closely related species, *Actinomadura keratinilytica* WCC-2265[T], supported by the NJ, ME, and ML trees with 100% bootstrap values. Based on the phylogenetic species concept, the three *Actinobacteria* isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) were identified as *Actinomadura keratinilytica*. Sriyapai et al. (2011) and Taibi et al. (2012) reported that *A. keratinilytica* strains were xylanase-producing bacteria, yet there was no information of xylanolytic activity from the type strain *A. keratinilytica* WCC-2265[T]. According to Sriyapai et al. (2011), *Actinomadura keratinilytica* S-14 was a thermophilic xylan-degrading bacterium isolated from compost, and this strain showed xylanolytic activity after being incubated at 55°C for 5 days. Taibi et al. (2012) reported another strain of *A. keratinilytica*, Cpt20, isolated from poultry compost, was a thermostable xylanase producer. Its xylanase demonstrated high thermostability at 60°C for 5 days, with 2 and 1 h of half-life times at 90 and 100°C, respectively. Moreover, the xylanase produced by strain Cpt20 was affected by the presence of metal ions Mn^{2+}, Ca^{2+}, and Cu^{2+} and inhibited by Hg^{2+}, Zn^{2+}, and Ba^{2+}.
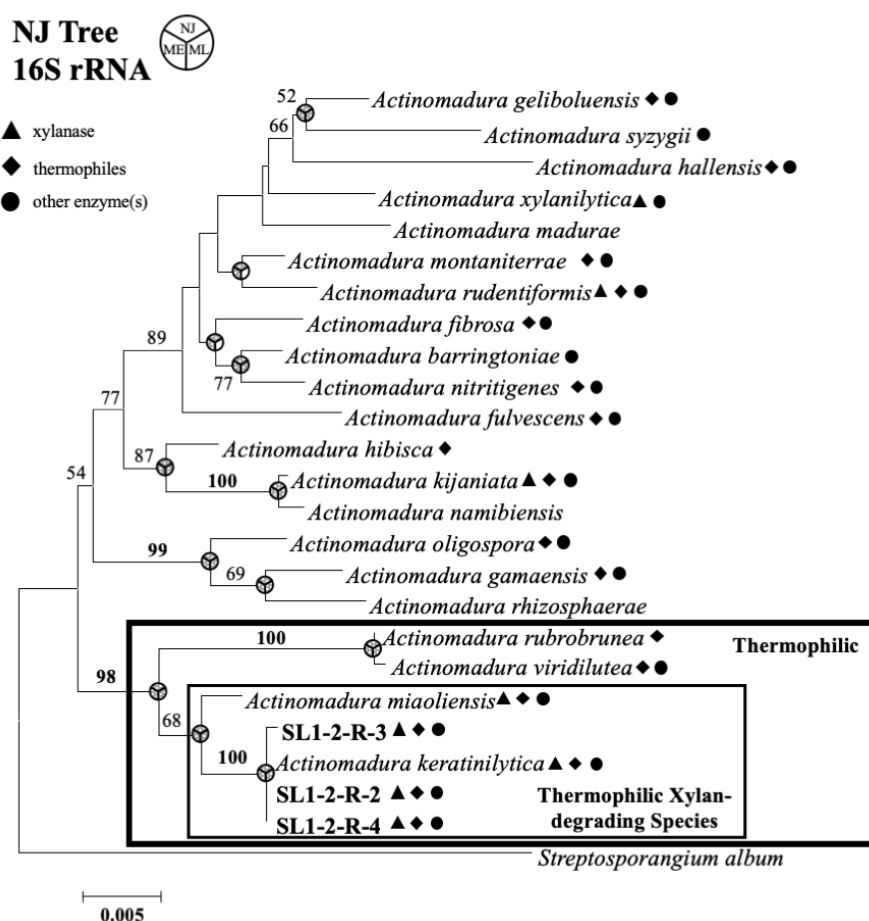

Figure 2. The neighbor-joining tree of three *Actinobacteria* isolates and related *Actinomadura* species based on 1,335 aligned positions of the 16S rRNA gene sequences. *Streptosporangium album* DSM 43023[T] was used as an outgroup. Closed circles indicate the branches of the tree that were also found using maximum likelihood and minimum evolution tree constructions. The numbers at the branch nodes represent the percentage of bootstrap values with 1,000 replicates. Only bootstrap values greater than 50% are shown.

Phylogenetic analyses showed that the three potential *Actinobacteria* isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4), the type strain *A. keratinilytica* WCC-2265[T], and *A. miaoliensis* BC 44T-5[T] formed a clade thermophilic xylan-degrading species, supported by the NJ, ME, and ML methods with 68% bootstrap values (Figure 2). Other strains from these two species were also reported as xylanase-producing bacteria by Sriyapai et al. (2018) and Taibi et al. (2012). The ability of the three *Actinobacteria* isolates used in this study (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) and the two *Actinomadura* species (*A. keratinilytica* and *A. miaoliensis*) to degrade xylan at high temperatures indicated their potential contribution in organic matter decomposition in nature. According to Trujillo and Goodfellow (2012), most of the *Actinomadura* species were soil inhabitants and played a major role in decomposition process in soil. In addition, some scientists reported that *Actinomadura keratinilytica* was also a potential producer of other enzymes, e.g. cellulase (Sriyapai et al. 2018), pectate lyase (Saoudi et al. 2015), keratinase (Habbeche et al. 2014), and amylase (Sukkhum et al. 2011), while *A. miaoliensis* was a potential producer of cellulase (Sriyapai et al. 2018). Based on this finding, the three potential isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) are potential as xylanase-producing *Actinobacteria*.

Three potential *Actinobacteria* isolates apparently formed a cluster with the thermophilic species *A. keratinilytica* WCC-2265[T], *A. miaoliensis* BC 44T-5[T], *A. rubrobrunea* NBRC 15275[T], and *A. viridilutea* IFO14480[T], supported by the NJ, ME, and ML methods, with 98% bootstrap values (Figure 2). *Actinomadura rubrobrunea* NBRC 15275[T] and *A. viridilutea* IFO 14480[T] were thermophilic species, yet their xylanolytic activities remain unknown. However, as reported by Elhoul et al. (2016), *A. viridilutea* was also a potential producer of keratinase.

The potential three *Actinobacteria* xylan-degrading isolates used in this study were obtained from soil samples collected around Cisolok geyser, indicating the soil around Cisolok geysers is a potential source of xylan-degrading *Actinomadura* species. According to Tseng et al. (2009), and Trujillo and Goodfellow (2012), *Actinomadura miaoliensis* BC 44T-5[T] and *A. rubrobrunea* NBRC 15275[T] were also isolated from soil samples. However, some of the closely related species of these three isolates were isolated from extreme habitats, such as bovine hoof keratin bait in bovine manure compost (*Actinomadura keratinilytica* WCC-2265[T]; Puhl et al. 2009) and desert soil (*A. viridilutea* IFO 14480[T]; Agre and Guzeva 1975).

The *Actinomadura* species used in the phylogenetic tree (Figure 2), were mostly isolated from soil (Tomita et al. 1989; Wink et al. 2003; Lee and Jeong 2006; Tseng et al. 2009; Trujillo and Goodfellow 2012; Zucchi et al. 2013; Zhao et al. 2015; Malisorn et al. 2018); some species were associated with plants (Rachniyom et al. 2015; Rachniyom et al. 2018); one found in biofilters consisting of tree bark compost (Songsumanus et al. 2016); and some were pathogens to animals, including humans (Trujillo and Goodfellow 2012). Information on xylanase-producing

Actinomadura species remains limited, although various species, such as *A. keratinilytica* Cpt20 (Taibi et al. 2012), *A. miaoliensis* TF1 (Sriyapai et al. 2018), *A. xylanilytica* BK147[T] (Zucchi et al. 2013), *A. rudentiformis* HMC1[T] (le Roes and Meyers 2007), and *A. kijaniata* SCC 1256[T] (Horan and Brodsky 1982), have been reported to display xylanolytic activity. Some *Actinomadura* species produced other enzymes, such as cellulase (Trujillo and Goodfellow 2012, Sriyapai et al. 2018), amylase (Horan and Brodsky 1982; Lee and Jeong 2006; Sazak et al. 2012; Trujillo and Goodfellow 2012; Rachniyom et al. 2015; Zhao et al. 2015; Abagana et al. 2016; Songsumanus et al. 2016; Rachniyom et al. 2018), gelatinase (Horan and Brodsky 1982; Mertz and Yao 1986; Zucchi et al. 2013), and keratinase (Puhl et al. 2009; Trujillo and Goodfellow 2012; Habbeche et al. 2014; Elhoul et al. 2016), which demonstrated that *Actinomadura* species are potential enzymes producers. Most of *Actinomadura* species were mesophilic bacteria, which can grow well at temperatures between 25°C to 40°C. However, some *Actinomadura* species such as *A. formosensis, A. rubrobrunea*, *A. viridilutea*, and *A. keratinilytica* were considered thermophiles, which grown optimally at temperatures between 45°C to 65°C (Trujillo and Goodfellow 2012).

Based on our previous study (Syafitri et al. 2019), the three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) belong to *Actinomadura keratinilytica*. However, the xylanase activity from the type strain of *Actinomadura keratinilytica* WCC-2265[T] was not yet reported. Therefore, the confirmation of xylan-degrading ability for the three potential isolates along with the type strain of *Actinomadura keratinilytica* WCC-2265[T] was conducted in this study.

Xylan-degrading ability of the potential *Actinobacteria* isolates and type strain *A. keratinilytica* NBRC 105837[T]

As there was no information about the ability of the type strain *Actinomadura keratinilytica* NBRC 105837[T] (=WCC-2265[T]) to degrade xylan, the xylan-degrading ability of the type strain was examined in the present study. The ability of the type strain *Actinomadura keratinilytica* NBRC 105837[T] to hydrolyze xylan was examined together with the three potential isolates from this study (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4). The xylan-degrading ability was examined using 0.5% xylan and 0.1% RBB-xylan as substrates at various temperatures. The result of xylan-degrading ability of the three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) and the type strain *A. keratinilytica* NBRC 105837[T] in Mm medium with the addition of 0.1% RBB-xylan at temperatures of 45 to 60°C is shown in Figure 3. The result of xylan-degrading ability of the type strain *A. keratinilytica* NBRC 105837[T] in Mm medium with the addition of 0.5% xylan at 45 to 60°C is shown in Figure 4.

After 3 days of incubation on 0.1% RBB-xylan plates at temperatures of 45 to 60°C, the three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) were confirmed for positive xylan-degrading ability (Figure 3). As mentioned by Jeffrey et al. (2011), the presence of clear zones around

colonies on a medium containing RBB-xylan indicating the production of extracellular xylanases. In addition, a 0.1% RBB-xylan medium could be used in the preliminary screening of xylanase for actinomycetes. The RBB-xylan is a soluble chromogenic substrate used to detect endo-1,4-β-xylanase based on changes in the medium's color due to the enzymatic reactions (Brennan et al. 2004; Juturu and Wu 2012; Skovgaard and Jørgenson 2013).
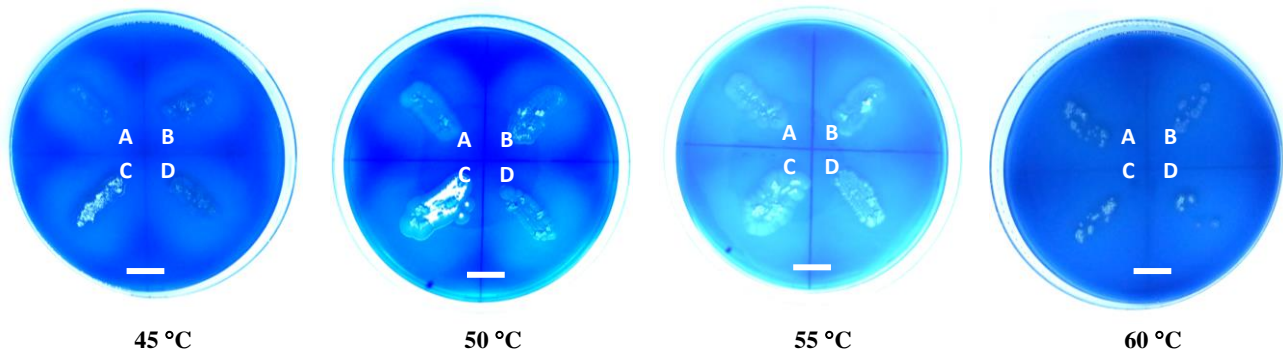

Figure 3. Examination of xylan-degrading ability for four *Actinobacteria* isolates grown on minimal medium supplemented with 0.1% RBB-xylan and incubated for 3 days: A. SL1-2-R-2, B. SL1-2-R-3, C. SL1-2-R-4, and D. *Actinomadura keratinilytica* NBRC 105837[T]. Bar = 1 cm
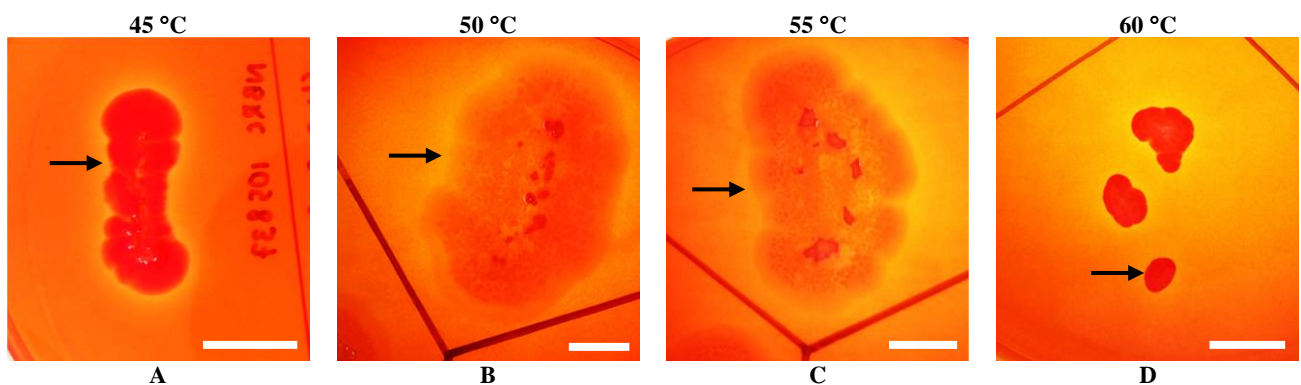
Figure 4. Clear zone formation in *Actinomadura keratinilytica* NBRC 105837[T] (A-D) grown on minimal medium with addition of 0.5% xylan. Bar = 1 cm
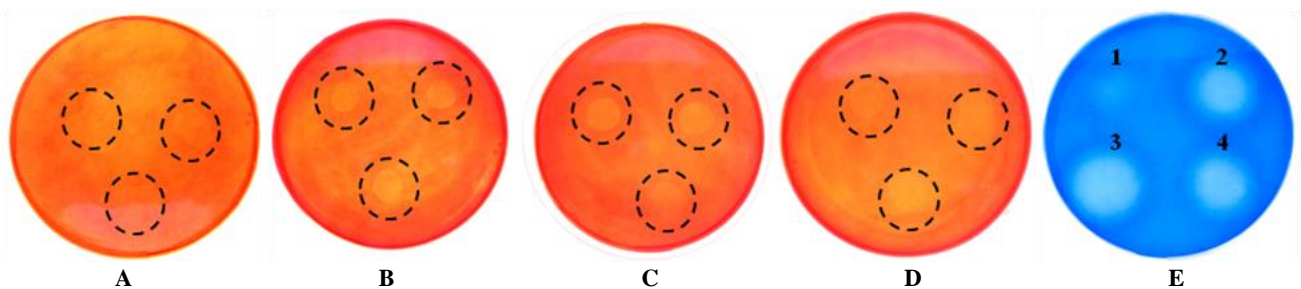

Figure 5. Clear zone formation of culture supernatants containing crude xylanase from three potential isolates and type strain on Mm medium containing 0.5% xylan (A-D): A. SL1-2-R-2, B. SL1-2-R-3, C. SL1-2-R-4, D. NBRC 105837[T]; and E. 0.1% RBB-xylan: (1) SL1-2-R-2, (2) SL1-2-R-3, (3) SL1-2-R-4, and (4) NBRC 105837[T] after incubation at 60°C for 24 hours. Arrow indicated clear zone.

The ability of culture supernatants containing crude enzymes of xylanase from three potential isolates and type strain *A. keratinilytica* NBRC 105837[T] to hydrolyze 0.5% xylan and 0.1% RBB-xylan on Mm medium were evaluated at various temperatures (45, 50, 55, and 60°C). The crude enzymes from three potential isolates and type strain *A. keratinilytica* NBRC 105837[T] showed the ability to hydrolyzed 0.5% xylan and 0.1% RBB-xylan on Mm medium at various temperatures (45, 50, 55, and 60°C), indicated by the formation of clear zones. Figure 5 showed the clear zones formation of crude enzymes from three isolates and type strain at temperature of 60°C. The results showed the culture supernatants contained extracellular xylanase that could hydrolyzed substrates at high temperatures ranging from 45 to 60°C. McCarthy et al. (1985) reported that the increasing of incubation temperatures for culture supernatant might be associated with thermostability of xylanase.

The results of extracellular xylanase production in this study might demonstrate that xylanase production is an inducible activity in microorganisms. Seven days of incubation on Mm broth containing 0.5% xylan might be addressed to late growth period of cultures, thus indicating the culture might produce high quantities of xylanolytic enzymes. According to the previous studies, the production of xylanase was induced by incubating microorganism on xylan-containing medium (McCarthy et al. 1985), due to the abundant of xylan, xylose, or xylooligosaccharides on culture medium (Khucharoenphaisan et al. 2010). Xylanase production was highly associated with growth rate of actinomycetes (Dou et al. 2019), and production of extracellular endoxylanase was associated with degradation of xylan (McCarthy et al. 1985). Furthermore, study regarding the measurement of reducing sugar released from tested substrates is required to obtain the types of xylanases produced by tested microorganisms.

In addition, the cellulose-degrading ability for three potential isolates at high temperatures were also reported in the previous study. The ability of three potential isolates to produce xylanolytic and cellulolytic enzymes might indicate the possession of multicomplex xylanase-cellulase system. Setyaningsih et al. (2019) reported that the three potential isolates were able to degrade CMC and MCC as substrates at various temperatures (45, 50, 55, and 60°C) due to the production of cellulolytic enzyme. Pohlschröder et al. (1994) reported another *Actinobacteria*, namely *Clostridium papyrosolvens* C7 possessed multiple complexes of cellulase-xylanase system that correspond to degradation of cellulose and xylan polymers.

Our study also revealed that the type strain *A. keratinilytica* NBRC 105837[T] was able to grow and showed xylan-degrading ability in both 0.1% RBB-xylan (Figure 3) and 0.5% xylan (Figure 4) as substrates at temperatures of 45, 50, 55, and 60°C. According to the previous study by Puhl et al. (2009), the *A. keratinilytica* WCC-2265[T] was able to grow at temperatures of 30 to 55°C with optimum growth at 45°C. This study provides new information regarding the growth ability of the type strain *A.*

keratinilytica NBRC 105837[T] at temperature of 60°C and its xylan-degrading ability at 45 to 60°C. The study regarding the thermostability of the xylanase produced by the three potential isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) is needed to be further examined.

This study showed that the use of the Congo red staining method to detect extracellular xylanase on Mm medium containing 0.5% xylan formed a clear zone only around the colonies (Figure 1 and Figure 4). As shown in Figure 1 and Figure 4, the clear zone was faintly visible and thus might be difficult to observe, to distinguish, and to document. In contrast, it can be seen in Figure 3 that the result for the detection of xylan-degrading ability using the RBB-xylan showed an unambiguous clear zone around the colonies; thus, the clear zone was easier to distinguish compared with that obtained using the Congo red method. The similar results were observed by Meddeb-Mouelhi et al. (2014) while detecting the xylan-degrading ability by using Congo red staining method on minimal medium containing 0.5% xylan and 0.1% RBB-xylan. The detection of xylan-degrading ability by using Congo red staining showed an ambiguous clear zone around bacterial colonies, while the detection by using chromogenic medium showed an obvious clear zone. Thus, the use of medium plates containing RBB-xylan is promising rather than Congo red staining method for detection of xylan-degrading ability.

Observation of morphological properties

According to Barka et al. (2016), the main morphological features that characterize *Actinobacteria* are the presence or absence of mycelial and aerial hyphae, the mycelium color, the types of spore chains and spores, the spore chain length, and the production of melanoid pigments. Trujillo and Goodfellow (2012) reported that *Actinomadura* mainly formed branched non-fragmenting substrate mycelium, with an aerial mycelium that was either absent or developed with harboring arthrospores, and with straight, hooked, or irregular spirals of spore chains.

The morphological properties of three *Actinobacteria* isolate grown on modified Bennett's gellan after 14 days of incubation at 45°C are shown in Figure 6. The three isolates (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) produced non-fragmenting branched substrate hyphae with aerial hyphae borne directly from the substrate hyphae. The aerial hyphae differentiated into straight or flexuous spore chains (Figure 6). These morphological properties were consistent with those of *A. keratinilytica* WCC-2265[T]. According to Puhl et al. (2009), *A. keratinilytica* WCC-2265[T] possessed non-fragmenting with branched substrate hyphae and with branched aerial mycelia. The aerial mycelia differentiated into straight or flexuous chains of up to 15 spiny spores after 48 h of incubation at 45°C on keratin agar. Thus, the three *Actinomadura keranitilytica* strains in this study (SL1-2-R-2, SL1-2-R-3, and SL1-2-R-4) were xylan-degrading *Actinobacteria*, that shared the same xylan-degrading ability and morphological characters to the type strain *A. keratinilytica* NBRC 105837[T] (=WCC-2265[T]).
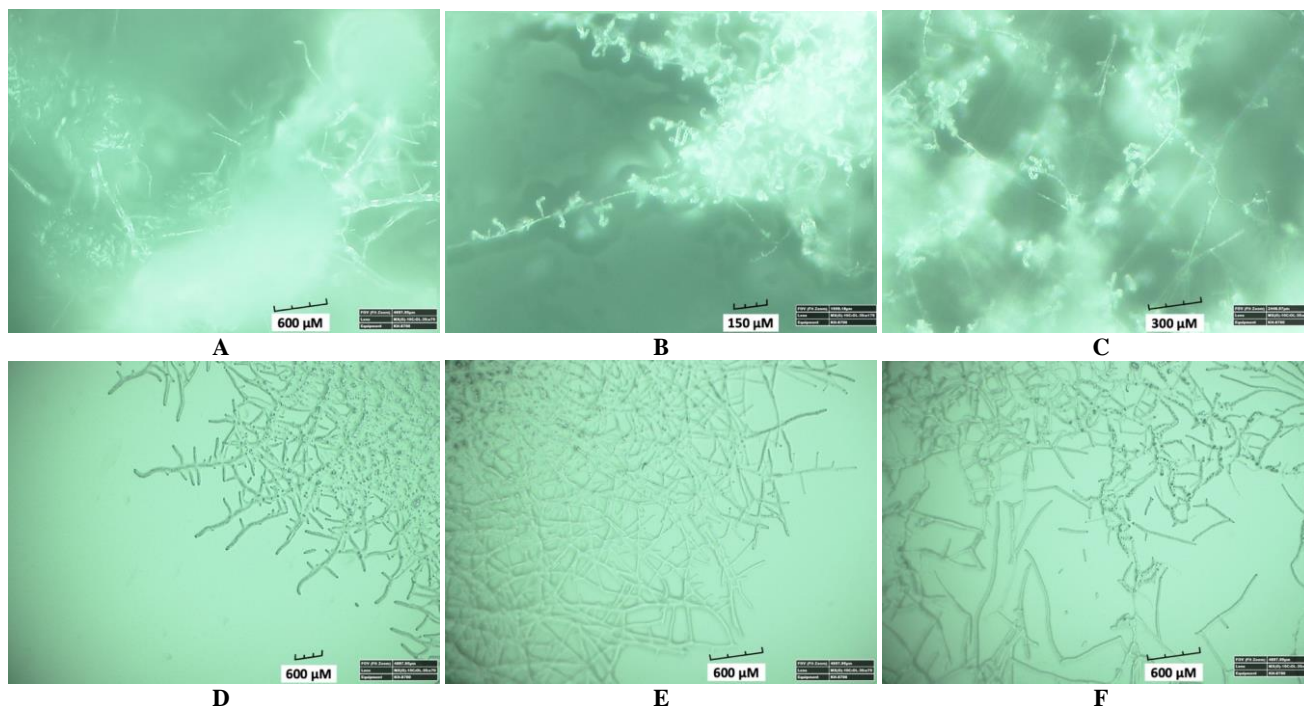
Figure 6. Morphological characteristics of the three *Actinomadura keratinilytica* isolates after being incubated for 14 days at 45°C. Structure of aerial mycelia on modified Bennett's gellan (A-C): A. SL1-2-R-2, B. SL1-2-R-3, C. SL1-2-R-4; structure of substrate mycelia on modified Bennett's agar (D-F): D. SL1-2-R-2, E. SL1-2-R-3, and F. SL1-2-R-4.

ACKNOWLEDGEMENTS

REFERENCES

Abagana AY, Sun P, Liu C, Cao T, Zheng W, Zhao S, Xiang W, Wang X. 2016. *Actinomadura gamaensis* sp. nov., a novel actinomycete isolated from soil in Gama, Chad. Antonie Leeuwenhoek 109 (6): 833-839.

Agre NS, Guzeva LN. 1975. New actinomycetes genus: *Excellospora* gen. nov. Mikrobiologiya 44 (3): 518-523.

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, van Wezel GP. 2016. Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol Mol Biol Rev 80 (1): 1-43.

Beg QK, Kapoor M, Mahajan L, Hoondal GS. 2001. Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol 56 (3-4): 326-338.

Brennan Y, Callen WN, Christoffersen L, Dupree P, Goubet F, Healey S, Hernández M, Keller M, Li K, Palackal N, Sittenfeld A, Tamayo G, Wells S, Hazlewood GP, Mathur EJ, Short JM, Robertson DE, Steer BA. 2004. Unusual microbial xylanases from insect guts. Appl Environ Microbiol 70 (6): 3609-3617.

Chae HJ, Joo H, In M-J. 2001 Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresour Technol 76 (3): 253-258.

Dou T-Y, Chen J, Hao Y-F, Qi X. 2019. Effects of different carbon sources on enzyme production and ultrastructure of *Cellulosimicrobium cellulans*. Curr Microbiol 76 (3): 355-360.

Elhoul MB, Jaouadi NZ, Rekik H, Benmrad MO, Mechri S, Moujehed E, Kourdali S, Hattab ME, Badis A, Bejar S, Jaouadi B. 2016. Biochemical and molecular characterization of new keratinoytic protease from *Actinomadura viridilutea* DZ50. Intl J Biol Macromol 92: 299-315.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17 (6): 368-376.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39 (4): 783-791.

Goodfellow M, Trujillo ME. 2012. Family III. Thermomonosporaceae Rainey, Ward-Rainey and Stackebrandt 1997, 486 emend. Zhang, Wang and Ruan 2001, 381 emend. Zhi, Li and Stackebrandt 2009, 600. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K, Ludwig W, Whitman WB. (eds) Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer, New York.

Goodfellow M, Williams ST. 1983. Ecology of actinomycetes. Annu Rev Microbiol 37: 189-216.

Habbeche A, Saoudi B, Jaouadi B, Haberra S, Kerouaz B, Boudelaa M, Badis A, Ladjama A. 2014. Purification and biochemical characterization of a detergent-stable keratinase from a newly thermophilic actinomycete *Actinomadura keratinilytica* strain Cpt29 isolated from poultry compost. J Biosci Bioeng 117 (4): 413-421.

Hakobyan L, Gabrielyan L, Trchounian A. 2012. Yeast extract as an effective nitrogen source stimulating cell growth and enhancing hydrogen photoproduction by *Rhodobacter sphaeroides* strains from mineral springs. Int J Hydrog Energy 37 (8): 6519-6526.

Horan AC, Brodsky BC. 1982. A Novel Antibiotic-Producing *Actinomadura*, *Actinomadura kijaniata* sp. nov. Intl J Syst Evol Microbiol 32 (2): 195-200.

Jeffrey LSH, Norzaimawati AN, Rosnah H. 2011. Prescreening of bioactivities from actinomycetes isolated from forest peat soil of Sarawak. J Trop Agric Food Sci 39 (2): 245-253.

Jones KL. 1949. Fresh isolates of *Actinomycetes* in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J Bacteriol 57 (2): 141-145.

Juturu V, Wu JC. 2012. Microbial xylanases: engineering, production and industrial applications. Biotechnol Adv 30 (6): 1219-1227.

Khucharoenphaisan K, Tokuyama S, Kitpreechavanich V. 2010. Purification and characterization of a high-thermostable β-xylanase from newly isolated *Thermomyces lanuginosus* THKU-49. Mycoscience 51 (6): 405-410.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16 (2): 111-120.

Kulkarni N, Shendye A, Rao M. 1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol Rev 23 (4): 411-456.

Kumar S, Stecher G, Tamura T. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33 (7): 1870-1874.

Kumar V, Dangi AK, Shukla P. 2018. Engineering thermostable microbial xylanases toward its industrial applications. Mol Biotechnol 60 (3): 226-235.

le Roes M, Meyers PR. 2007. *Actinomadura rudentiformis* sp. nov., isolated from soil. Int J Syst Evol Microbiol 57 (1): 45-50.

Lee LS, Goh KM, Chan CS, Tan GYA, Yin W-F, Chong CS, Chan K-G. 2018. Microbial diversity of thermophiles with biomass deconstruction potential in a foliage-rich hot spring. Microbiology Open 7 (6) :e00615. DOI: 10.1002/mbo3.615

Lee SD, Jeong HS. 2006. *Actinomadura hallensis* sp. nov., a novel actinomycete isolated from Mt. Halla in Korea. Intl J Syst Evol Microbiol 56 (1): 259-264.

MacKenzie CR, Williams RE. 1984. Detection of cellulase and xylanase activity in isoelectric-focused gels using agar substrate gels supported on plastic film. Can J Microbiol 30 (12): 1522-1525.

Malisorn K, Kanchanasin P, Phongsopitanun W, Tanasupawat S. 2018. *Actinomadura rhizosphaerae* sp. nov., isolated from rhizosphere soil of the plant *Azadirachta indica*. Intl J Syst Evol Microbiol 68 (9): 3012-3016.

Mawarid AT, Sjamsuridzal W, Yabe S, Santoso I, Ningsih F, Nurlaili DG, Oetari A, Yokota A. 2016. Polyphasic identification of a thermophilic bacterium from geyser of Cisolok, Indonesia. AIP Conf Proc 1729: 020067. DOI: 10.1063/1.4946970.

McCarthy AJ, Peace E, Broda P. 1985. Studies on the extracellular xylanase activity of some thermophilic actinomycetes. Appl Microbiol Biotechnol 21 (3-4): 238-244.

Meddeb-Mouelhi F, Moisan JK, Beauregard M. 2014. A comparison of plate assay methods for detecting extracellular cellulase and xylanase activity. Enzyme Microb Technol 66: 16-19.

Mertz FP, Yao RC. 1986. *Actinomadura oligospora* sp. nov. the producer of a new polyether antibiotic. Intl J Syst Bacteriol 36 (2): 179-182.

Moreira LRS, Filho EXF. 2016. Insights into the mechanism of enzymatic hydrolysis of xylan. Appl Microbiol Biotechnol 100 (12): 5205-5214.

Ningsih F, Yokota A, Sakai Y, Nanatani K, Yabe S, Oetari A, Sjamsuridzal W. 2019. *Gandjariella thermophila* gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*, isolated from forest soil in a geothermal area. Intl J Syst Evol Microbiol 69 (10): 3080-3086.

Pohlschröder M, Leschine SB, Canale-Parola E. 1994. Multicomplex cellulase-xylanase system of *Clostridium papyrosolvens* C7. J Bacteriol 176 (1): 70-76.

Puhl AA, Selinger LB, McAllister TA, Inglis GD. 2009. *Actinomadura keratinilytica* sp. nov., a keratin-degrading actinobacterium isolated from bovine manure compost. Intl J Syst Evol Microbiol 59 (4): 828-834.

Rachniyom H, Matsumoto A, Inahashi Y, Take A, Takahashi Y, Thamchaipenet A. 2018. *Actinomadura barringtoniae* sp. nov., an endophytic actinomycete isolated from the roots of *Barringtonia acutangula* (L.) Gaertn. Intl J Syst Evol Microbiol 68 (5): 1584-1590.

Rachniyom H, Matsumoto A, Indananda C, Duangmal K, Takahashi Y, Thamchaipenet A. 2015. *Actinomadura syzygii* sp. nov., an endophytic actinomycete isolated from the roots of a jambolan plum tree (*Syzygium cumini* L. Skeels). Intl J Syst Evol Microbiol 65 (6): 1946-1952.

Rzhetsky A, Nei M. 1992. A simple method for estimating and testing minimum-evolution trees. Mol Biol Evol 9 (5): 945-967.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4 (4): 406-425.

Saoudi B, Habbeche A, Kerouaz B, Haberra S, Romdhane ZB, Tichati L, Boudelaa M, Belghith H, Gargouri A, Ladjama A. 2015. Purification and characterization of a new thermoalkaliphilic pectate lyase from *Actinomadura keratinilytica* Cpt20. Process Biochem 50 (12): 2259-2266.

Sazak A, Camas M, Spröer C, Klenk H-P, Sahin N. 2012. *Actinomadura geliboluensis* sp. nov., isolated from soil. Intl J Syst Evol Microbiol 62 (8): 2011-2017.

Setyaningsih PP, Ningsih F, Rachmania MK, Syafitri WA, Sari DCAF, Yabe S, Yokota A, Oetari A, Sjamsuridzal W. 2019. Cellulolytic enzyme-producing thermophilic *Actinobacteria* isolated from the soil of Cisolok Geysers, West Java, Indonesia. Biodiversitas 20 (11): 3134-3141.

Shivlata L, Satyanarayana T. 2015. Thermophilic and alkaliphilic *Actinobacteria*: biology and potential applications. Front Microbiol 6: 1014.

Singh SP, Shukla RJ, Kikani BA. 2013. Molecular diversity and biotechnological relevance of thermophilic *Actinobacteria*. In: Satyanarayana T, Littlechild J, Kawarabayasi Y. (eds.). Thermophilic Microbes in Environmental and Industrial Biotechnology: Biotechnology of Thermophiles, 2nd ed. Springer, New York.

Skovgaard PA, Jørgenson H. 2013. Influence of high temperature and ethanol on thermostable lignocellulolytic enzymes. J Ind Microbiol Biotechnol 40 (5): 447-456.

Songsumanus A, Kudo T, Ohkuma M, Phongsopitanun W, Tanasupawat S. 2016. *Actinomadura montaniterrae* sp. nov., isolated from mountain soil. Intl J Syst Evol Microbiol 66 (9): 3310-3316.

Sriyapai P, Chansiri K, Sriyapai T. 2018. Isolation and characterization of polyester-based plastics-degrading bacteria from compost soils. Microbiology 87 (2): 290-300.

Sriyapai T, Somyoonsap P, Matsui K, Kawai F, Chansiri K. 2011. Cloning of a thermostable xylanase from *Actinomadura* sp. S14 and its expression in *Escherichia coli* and *Pichia pastoris*. J Biosci Bioeng 111 (5): 528-536.

Sukkhum S, Tokuyama S, Kongsaeree, Tamura T, Ishida Y, Kitpreechavanich V. 2011. A novel poly (L-lactide) degrading thermophilic actinomycetes, *Actinomadura keratinilytica* strain T16-1 and pla sequencing. Afr J Microbiol Res 5 (18): 2575-2582.

Suzuki H, Sawai Y, Suzuki T, Kawai K. 2003. Purification and characterization of an extracellular β-agarase from *Bacillus* sp. MK03. J Biosci Bioeng 95 (4): 328-334.

Syafitri WA, Ningsih F, Setyaningsih PP, Rachmania MK, Sari DCAF, Yabe S, Yokota A, Oetari A, Sjamsuridzal W. 2019. Screening for amylolytic activity and characterization of thermophilic *Actinobacteria* isolated from a Geothermal Area in West Java, Indonesia. Biodiversitas 20 (7): 1929-1938.

Taibi Z, Saoudi B, Boudelaa M, Trigui H, Belghith H, Gargouri A, Ladjama A. 2012. Purification and biochemical characterization of highly thermostable xylanase from *Actinomadura* sp. strain Cpt 20 isolated from poultry compost. Appl Biochem Biotechnol 166 (3): 663-679.

Tomita K, Nishio M, Saitoh K, Yamamoto H, Hoshino Y, Ohkuma H, Konishi M, Miyaki T, Oki T. 1989. Pradmicins A, B, and C: new antifungal antibiotics I. Taxonomy, production, isolation, and physicochemical properties. J Antibiot 43 (7): 755-762.

Trujillo ME, Goodfellow M. 2012. Genus III. *Actinomadura* Lechevalier and Lechevalier 1970a, 400[AL] emend. Kroppenstedt, Stackebrandt and Goodfellow 1990, 156. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K-I, Ludwig W, Whitman WB. (eds.), Bergey's Manual of Systematic Bacteriology, 2nd ed, Volume 5: *Actinobacteria*, part B. Springer, New York.

Tseng M, Yang SF, Hoang KC, Liao HC, Yuan GF, Liao CC. 2009. *Actinomadura miaoliensis* sp. nov., a thermotolerant polyester-degrading actinomycete. Int J Syst Evol Microbiol 59 (3): 517-520.

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

Walia A, Guleria S, Mehta P, Chauhan A, Parkash J. 2017. Microbial xylanases and their industrial application in pulp and paper biobleaching: a review. 3 Biotech 7 (1): 11.

Wink J, Kroppenstedt RM, Seibert G, Stackebrandt E. 2003. *Actinomadura namibiensis* sp. nov. Intl J Syst Evol Microbiol 53 (3): 721-724.

Yokota A, Ningsih F, Nurlaili DG, Sakai Y, Yabe S, Oetari A, Santoso I, Sjamsuridzal W. 2016. *Paenibacillus cisolokensis* sp. nov., isolated from litter of a geyser. Intl J Syst Evol Microbiol 66 (8): 3088-3094.

Zhao J, Guo L, Sun P, Han C, Bai L, Liu C, Li Y, Xiang W, Wang X. 2015. *Actinomadura jiaoheensis* sp. nov. and *Actinomadura sporangiiformans* sp. nov., two novel actinomycetes isolated from muddy soil and emended description of the genus *Actinomadura*. Antonie van Leeuwenhoek 108 (6): 1331-1339.

Zucchi TD, Kim B-Y, Bonda ANV, Goodfellow M. 2013. *Actinomadura xylanilytica* sp. nov., an actinomycete isolated from soil. Int J Syst Evol Microbiol 63 (2): 576-580.