

Isolation and Identification of Actinomycetes Associated with Moss on the Surface of the Borobudur Temple Stone

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Abstract. Mosses growing on the surface of the Borobudur Temple will affect the aesthetic value of the temple. Interaction between moss and actinomycetes may trigger the growth of moss that can cause an increase in biodeterioration of stone. The purpose of this study was to isolate and identify the actinomycetes associated with moss on the surface of decayed stone of Borobudur Temple as well as to assess their ability for phosphate solubilizing. Actinomycetes were isolated using serial dilution method and were identified based on 16S rRNA gene sequences. A total of 37 actinomycetes were isolated from three sampling sites. The isolates found belong to five genera (*Gordonia*, *Microbacterium*, *Micromonospora*, *Nocardia*, and *Streptomyces*) and distributed among four families (Microbacteriaceae, Micromonosporaceae, Nocardiaceae, and Streptomycetaceae). Isolates of actinomycetes composed of 19 *Streptomyces* Group and 18 Rare Actinomycetes Group. Nineteen isolates (51.35%) were identified as genus *Streptomyces*. Seventeen isolates (45.94%) showed abilities to release soluble phosphate and most of the isolates belong to the genus *Streptomyces*. The isolates have been collected will be deposited to Indonesian Culture Collection (InaCC) to enrich the collection of actinomycetes from ancient stone in Indonesia and will be used as a source of reference material research, taxonomic, or as a source for further study.

Keywords: Actinomycetes; Borobudur Temple; Moss; Stone; Soluble Phosphate

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INTRODUCTION

Borobudur Temple is one of the Buddhist monuments located in Magelang Regency in the central part of Java, Indonesia (Hermawan, et al., 2016). Borobudur Temple was composed using andesite materials (Haldoko et al., 2014). The andesite stones of Borobudur Temple have different characteristics (hardness, porosity, and alkalinity) that affect its susceptibility to biodeterioration (Haldoko et al., 2014). Degradation of the ancient stone may be caused by the growth and the activity of living organisms such as bacteria (including actinomycetes), fungi, archaea, algae, mosses, and lichens (Purawijaya & Priyantika, 2013). Characteristic of the stone and the environmental condition influence the extent of microorganism colonization and biodeterioration process. High rainfall will increase the moisture of the stone, thus trigger the growth of microorganisms. Many studies have been reported that microorganisms were found on the surface of stone monument (Keshari & Adhikary, 2014; Pinheiro et al., 2018). Some microorganisms may grow on the surface (epilithic) or may penetrate the rock pore system and grow in the crack and pores of the stone (endolithic) (Gaylarde, Gaylarde, & Neilan, 2012).

Mosses are one of most found organism on the surface of Borobudur Temple. Although mosses have

been reported not to be the main cause of deterioration on Borobudur stones (Purawijaya & Priyantika, 2013), but if mosses grow on a large scale on the surface of stone, it will affect the aesthetic value of the Borobudur Temple. Therefore, it is important to preserve the Borobudur Temple from further natural destruction for our future generation. Interaction between moss and other endolithic microorganisms such as actinomycetes can enhance the rate of biodegradation.

Actinomycetes are Gram-positive bacteria with high Guanine and Cytosine content. Actinomycetes are divided into two groups, the *Streptomyces* Group and Rare Actinomycetes Group (non-*Streptomyces* strains). Actinomycetes are interesting to study because of their ability to produce various secondary metabolites and to form a mycelium that can grow in crack and pores of the stone (Abdulla et al. 2008). Actinomycetes have the ability to utilize organic matter present in stone as a carbon source. Actinomycetes that are associated with moss on the surface of stone may trigger the growth of moss and cause an increase in biodeterioration of stone. Actinomycetes may have an important role in providing nutrients for moss growth. Actinomycetes have been reportedly isolated from various types of historical monuments (Abdelhafez, El-Wekeel, Ramadan, & Abed-Allah, 2012; H.

Li, Lan, Katayama, Gu, & Wang, 2010; Q. Li, Zhang, He, & Yang, 2016; Pinheiro et al., 2018).

Study about actinomycetes isolated from Borobudur Temple stone has never been reported before, especially regarding its association with moss that overgrown the stone of Borobudur Temple. Therefore, the purpose of this study was to isolate and identify the actinomycetes associated with moss on the surface of the stone of Borobudur Temple and their ability in phosphate solubilizing. The isolation and identification of actinomycetes from decayed stone is important not only to understand their role in decayed stone of Borobudur Temple, but is also as a source for taxonomical study. The actinomycetes have been collected will be deposited to Indonesian culture collection (InaCC) to enrich the collection of actinomycetes from ancient stone in Indonesia. The isolates will be used as a source of reference material research, taxonomic, or as source for further research to the evaluation the effect of these actinomycetes to trigger the growth of mosses and to the evaluation their effect to cause damage to the stone.

METHODS

Samples Collection

The samples of mosses were collected from the surface of decayed stone of Borobudur Temple. The samples were collected using a spatula and put into plastic bags (Figure 1). The samples were obtained from three different tiers of Borobudur temple. They were collected from the base of the temple (A18BR1), the second tier of the temple (A18BR2), and top of temple (stupa) (A18BR3).



Figure 1. The stone overgrown with moss

Isolation of Actinomycetes Associated With Moss on the Surface of Decayed Stone of Borobudur Temple

Actinomycetes were isolated using serial dilution method. One gram of each sample was suspended in 10 mL sterile distilled water. The suspension was homogenized using vortex for 10 minutes and was shaken at room temperature using orbital shaker at 200 rpm for 15 minutes. One ml aliquot from the

suspension was transferred to a test tube containing 9 ml of sterile distilled water and was mixed well. The suspension was then diluted with sterile water up to 10^{-6} . About 100 μ L solution from each dilution was pipetted and was spread onto the plates of isolation medium. The medium used for isolation were Humic Acid Agar (HVA), and Actinomycetes Isolation Agar (AIA) supplemented with nalidixic acid (20 mg/L) and cycloheximide (50 mg/L). The plates were then incubated at 30°C for 7-14 days. Isolates of actinomycetes that have grown well and have different morphological characteristic were purified on Yeast Starch Agar (YSA) medium. The pure culture was kept in cryotube containing 10% glycerol at -80°C for further studies.

Molecular Identification of Actinomycetes and Phylogenetic Tree Analysis

Total genomic DNA was extracted using a modified method of Franco-Correa et al. (2010). The isolates of actinomycetes used as DNA template were prepared on YSA agar plate for seven days. The biomass of actinomycetes was then harvested by scraping off the mycelia and spore from a colony of actinomycetes from this media. The mycelia and spore were then put into microtube and incubated for overnight at -20°C. After incubation, the mycelia and spore of actinomycetes were cleaned three times with distilled water (500 μ l), and the samples were dispersed in lysis solution (Franco-Correa et al., 2010). Furthermore, partial 16S rRNA gene sequence was determined from the PCR-amplification fragment. The fragment of 16S rRNA gene sequence was amplified using universal primer 27F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3'). The PCR condition consisted of an initial denaturation at 94°C for 1 minute followed by 30 cycles of amplification (denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1 minute 30 seconds) (Putri, Lisdiyanti, & Kusmiati, 2018). Then the 16S rRNA gene fragment was sequenced by Macrogen®. The raw sequencing data were analyzed using Chromaspro program version 1.6. The complete fragments of 16S rRNA gene sequenced were compared with other sequences in the EzTaxon-e server (Kim et al., 2012). Furthermore, the 16S rRNA gene sequenced were aligned with the reference sequence of known species in a genus using CLUSTAL W. The phylogenetic tree of nucleotide sequences was constructed using the neighbor-joining distances method. The confidence of the clusters was assessed using a bootstrap analysis with 1000 replications.

In Vitro Screening of Phosphate-Solubilizing Actinomycetes

The isolates were cultivated on the minimal medium based on the Pikovskaya (PVK) medium. The medium contained 5 g/L Ca₃PO₄; 0.5 g/L yeast extract; 0,01 g/L FeCl₃.6H₂O; 10 g/L glucose; 0,2 g/L KCl; 0,1 g/L MgSO₄.7H₂O; 0,01 g/L MnSO₄.H₂O; 0,5 g/L (NH₄)₂.SO₄; and 18 g/L agar with pH 7 (Khan, Zaidi, & Musarrat, 2014). The actinomycetes were streaked (about 6 mm of diameter) on the surface of PVK medium then incubated at 30°C for 7-14 days and observed for halo formation. The positive result showed the formation of clear zones around the colony of actinomycetes on the culture plate.

RESULTS AND DISCUSSION

Isolation of Actinomycetes Associated with Moss on the Surface of Decayed Stone of Borobudur Temple

A total of 37 actinomycetes were isolated from three samples collection (Figure 2). The AIA medium supported higher count and more diversity of actinomycetes than HVA medium (Figure 3). The number of actinomycetes isolates varied in the three samples. The highest number of actinomycetes isolated from decayed stones at the third tier or Stupa (16 isolates). The pure isolates that were kept in cryotube with 10% glycerol at -80°C. Morphological observation obtained that actinomycetes had diverse colony colour. The colours observed were brown, black, yellow, pink, and white. Actinomycetes found from moss on the surface of decayed stone of Borobudur Temple were quite diverse. Abdulla et al. (2008) reported that the number and variation species of actinomycetes isolated from decay stone is higher than the sound stone. This is probably due to the nutrients available in decayed stoned are likely to be complex organic acid remains, the actinomycetes easier to utilize the nutrient a wide range of more complex and recalcitrant polymers such as polysaccharides and lignocellulose (Saini, Aggarwal, Sharma, & Yadav, 2015; Yeager et al., 2017). Abdulla et al. (2008) found 56 species of actinomycetes belonging to 15 genera from decayed stone. Haldoko et al. (2014) reported that the different characteristic of Borobudur temple stone causing differences in the level of damage and decay occurs.

Identification of Actinomycetes Based on 16 S rRNA Gene Sequence

The isolates of actinomycetes were divided into two groups (Streptomyces Group and Rare Actinomycetes Group). Streptomyces Group and Rare Actinomycetes Group were identified by analysis of par-

tial sequence of their 16S rRNA gene sequence and compared with known species in public database. A total of 19 isolates (51.35%) have belonged to Streptomyces Group, and 18 isolates (48.65%) have belonged to Rare Actinomycetes Group. The isolates were identified belong to five genera (Gordonia, Microbacterium, Micromonospora, Nocardia, and Streptomyces) and distributed among four families (Microbacteriaceae, Micromonosporaceae, Nocardiaceae, and Streptomycetaceae).

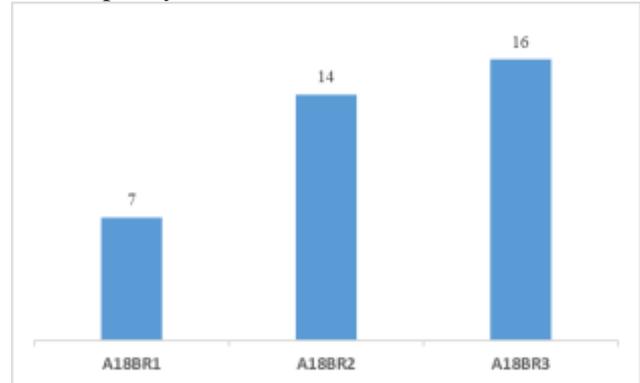


Figure 2. Number of actinomycetes isolated from moss on decayed stones of Borobudur Temple



Figure 3. Actinomycetes growth on AIA media plate for seven days and incubated at 30°C

Based on molecular characteristic, the isolates of Streptomyces Group included under genus *Streptomyces*. While, the isolates of Rare Actinomycetes Group included under four genera (*Microbacterium*, *Micromonospora*, *Gordonia*, and *Nocardia*) (Table 1). The most dominant Group of Rare Actinomycetes belong to genus *Micromonospora* in which nine isolates (Table 1). A large number of actinomycetes isolated from a decayed stone of Borobudur Temple belong to genus *Streptomyces* (51, 35%). This is possible because genus of *Streptomyces* is widely distributed. *Streptomyces* are widely distribution in environment and they grow quickly under conventional culture condition. By contrast, Rare Actinomycetes Group is generally characterized by slow growth and small colony (Hop et al., 2011). Fifty per cent of the actinomycetes isolated from decayed and sound stone samples taken from a tomb site at Tell Basta, Zagazig City, Egypt, was identified as *Streptomyces* (Abdulla et al., 2008).

Table 1. Diversity of actinomycetes associated with moss on decayed stones of Borobudur Temple

Group	Family	Genus	Number of isolates
Streptomyces	Streptomycetaceae	<i>Streptomyces</i>	19
Rare Actinomycetes	Microbacteriaceae	<i>Microbacterium</i>	7
	Micromonosporaceae	<i>Micromonospora</i>	9
	Nocardiaceae	<i>Gordonia</i>	1
		<i>Nocardia</i>	1
Total			37

Table 2. The Streptomyces Group isolated from moss on the surface of decayed stone of Borobudur Temple

Isolate code	Closest type strain		Similarity (%)
A18BR 1(2)	<i>Streptomyces chartreusis</i>	NBRC 12753(T)	99.25
A18BR 1(10)	<i>Streptomyces griseoluteus</i>	NBRC 13375	98.63
A18BR 1(11)	<i>Streptomyces termitum</i>	NBRC 13087(T)	99.43
A18BR 2(6)	<i>Streptomyces zhihengii</i>	YIM T102(T)	99.71
A18BR 2(9)	<i>Streptomyces polychromogenes</i>	NBRC 13072(T)	99.77
A18BR 2(12)	<i>Streptomyces avellaneus</i>	NBRC 13451(T)	99.63
A18BR 2(13)	<i>Streptomyces polychromogenes</i>	NBRC 13072	99.77
A18BR 3(5)	<i>Streptomyces bungoensis</i>	DSM 41781(T)	99.70
A18BR 3(6)	<i>Streptomyces griseoruber</i>	NRRL B-1818(T)	99.92
A18BR 3(8)	<i>Streptomyces tritici</i>	NRRL S-87	98.85
A18BR 3(9)	<i>Streptomyces bungoensis</i>	DSM 41781(T)	99.68
A18BR 3(13)	<i>Streptomyces tritici</i>	KY744943	98.79
A18BR 3(14)	<i>Streptomyces griseoluteus</i>	NBRC 13375	98.63
A18BR 3(15)	<i>Streptomyces neopeptinius</i>	KNF 2047(T)	99.19
A18BR 3(17)	<i>Streptomyces violaceorectus</i>	NBRC 13102(T)	98.74
A18BR 3(18)	<i>Streptomyces polychromogenes</i>	NBRC 13072(T)	99.77
A18BR 3(19)	<i>Streptomyces echinatus</i>	NBRC 12763(T)	98.96
A18BR 3(20)	<i>Streptomyces polychromogenes</i>	NBRC 13072(T)	99.76
A18BR 3(21)	<i>Streptomyces wuyuanensis</i>	CGMCC 4.7042(T)	100.00

A total of 19 isolates belong to the genus *Streptomyces* distributed among twelve species (Table 2). The isolates contained between 98.63-100% DNA homology to their closest type strain. A total of 18 isolates belong to Rare Actinomycetes Group distributed among eleven species (Table 3). The isolates contained between 98.08-100% DNA homology to their closest type strain. The dominant Group of Streptomyces belonged to the species *Streptomyces polychromogenes* (Table 2) and the dominant Group of Rare Actinomycetes belong to species *Micromonospora maritima* (Table 3).

The molecular identification was supported by the phylogenetic tree analysis based on a neighbour-joining tree. The 37 isolates sorted into four main clusters and the isolates confirmed were affiliation to four actinomycetes families (Microbacteriaceae, Micromonosporaceae, Nocardiaceae, and Streptomycetaceae). Nineteen isolates were affiliated within the

clustered of the family Streptomycetaceae (genus Streptomyces) (Figure 4). Seven isolates were affiliated within the cluster of the family Microbacteriaceae (genus Microbacterium) (Figure 5). Nine isolates were affiliated within the cluster of the family Micromonosporaceae (genus Micromonospora) (Figure 6). Two isolates were affiliated within the cluster of family Nocardiaceae (genera Nocardia and Gordonia) (Figure 6).

Some of the isolates showed different branch with other known species of the nearest genus on each cluster such as isolates (A18BR 3(13), A18BR 3(8), A18BR 3(17), and A18BR 3(15)) showed branch difference with the most closely related Streptomyces species (Figure 4). The isolate A18BR 2(15) also showed different branch from its nearest Microbacterium species neighbours (Figure 5). Further, a taxonomical study of the isolates compare with known species is needed.

Table 3. The Rare Actinomycetes Group isolated from moss on the surface of decayed stone of Borobudur Temple

Isolate code	Closest type strain		Similarity (%)
A18BR 1(1)	<i>Micromonospora maritima</i>	D10-9-5(T)	100.00
A18BR 1(3)	<i>Nocardia thailandica</i>	NBRC 100428(T)	98.08
A18BR 1(6)	<i>Micromonospora maritima</i>	D10-9-5	100.00
A18BR 1(8)	<i>Micromonospora sediminicola</i>	DSM 45794(T)	100.00
A18BR 2(1)	<i>Gordonia hongkongensis</i>	HKU50(T)	100.00
A18BR 2(2)	<i>Microbacterium arborescens</i>	DSM 20754	98.86
A18BR 2(3)	<i>Microbacterium arborescens</i>	DSM 20754(T)	99.01
A18BR 2(4)	<i>Microbacterium invictum</i>	DC-200(T)	98.81
A18BR 2(5)	<i>Microbacterium flavescens</i>	DSM 20643(T)	98.83
A18BR 2(7)	<i>Micromonospora maritima</i>	D10-9-5(T)	100.00
A18BR 2(8)	<i>Micromonospora mangrovi</i>	2803GPT1-18(T)	99.47
A18BR 2(10)	<i>Micromonospora maritima</i>	D10-9-5(T)	100.00
A18BR 2(11)	<i>Micromonospora mangrovi</i>	2803GPT1-18(T)	99.18
A18BR 2(15)	<i>Microbacterium aureliae</i>	JF-6(T)	98.50
A18BR 3(1)	<i>Microbacterium ginsengisoli</i>	DSM 18659(T)	99.01
A18BR 3(3)	<i>Microbacterium ginsengisoli</i>	DSM 18659(T)	98.97
A18BR 3(7)	<i>Micromonospora halophytica</i>	DSM 43171(T)	99.41
A18BR 3(11)	<i>Micromonospora maritima</i>	D10-9-5	100.00

Some species in this study have been reported successful isolated from various samples such as soil, plant, pollutant water, coastal, sea sediment, or human body (Carro et al., 2018; Luo, Hu, Peng, Zhang, & Wang, 2015; Wibberg et al., 2018; Klymyshin et al., 2013; Bourbour, Keikha, & Faghri, 2018; Tsang et al., 2016; Chen, Tang, Mori, & Wu, 2012; Kumar et al., 2016; Xie et al., 2016a). The isolates have been reported as a source of several secondary metabolites were *Micromonospora halophytica* (Carro et al., 2018), *Microbacterium ginsengisoli* (Palaniappan et al., 2010), *Streptomyces avellaneus* (Ramirez-Rodriguez et al., 2018), *Streptomyces bungoensis* (Michael et al., 2017), *Streptomyces chartreusis* (Wibberg et al., 2018), *Streptomyces echinatus* (Klymyshin et al., 2013), *Streptomyces griseoluteus* (Luo et al., 2015), *Streptomyces griseoruber* (Wang, Peng, Zhang, Hui, & Hu, 2014), *Streptomyces neopeptinius* (Goudjal et al., 2016), *Streptomyces polychromogenes* (Mitsukura, Sakamoto, Kubo, Yoshida, & Nagasawa, 2010), *Streptomyces termitum* (Sales et al., 2017), and *Streptomyces violaceorectus* (Fallah, 2018).

Two species were reported to cause diseases in human: they were *Gordonia hongkongensis* and *No-*

cardia thailandica (Bourbour, Keikha, & Faghri, 2018; Tsang et al., 2016). Both of the isolates belonged to family Nocardiaceae. Some species from this study also have been reported successfully isolated from coastal areas and mangroves sediments. The isolates were *Micromonospora sediminicola* isolated from marine sediment (Pittayakhajonwut et al., 2012), *Microbacterium aureliae* isolated from *Aurelia aurita*, the moon jellyfish (Kumar et al., 2016), *Micromonospora mangrove* (Xie et al., 2016a), and *Micromonospora maritima* (Xie et al., 2016b) isolated from mangrove sediment. Chemical compounds contained in the decayed rock of Borobudur Temple and many people visited this Temple may influences the genus/species of actinomycetes were founded. Haldoko et al. (2014) reported that the stone material of Borobudur Temple containing silicate (SiO₂), aluminium (Al₂O₃), iron Fe₂O₃, (FeO), calcium(CaO), magnesium (MgO), sodium (Na₂O), and potassium (K₂O). The stone overgrown with moss has higher potassium content, while SiO₂ content of stone overgrown with moss is lower than a stone that is not overgrown with moss (Haldoko et al., 2014).

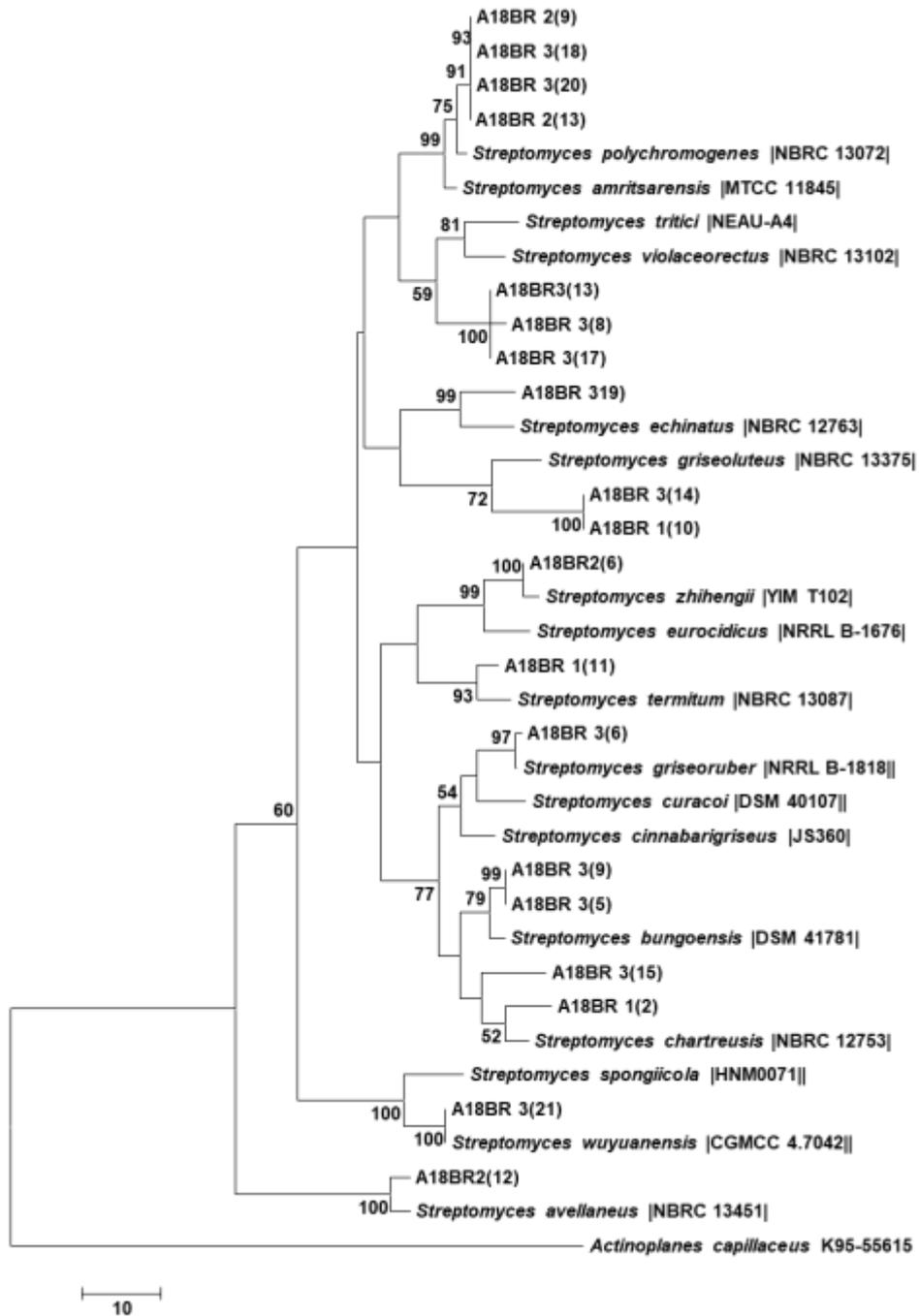


Figure 4. Neighbour-joining tree based on 16S rRNA gene sequences of actinomycetes isolated from mosses on the surface of decayed stone of Borobudur Temple and their closely related type strain within the family *Streptomycetaceae*. Bootstrap values are expressed as percentages 1000 replications. Bootstrap values >50 are shown at branch points. The bar represents ten substitutions per nucleotide position. *Actinoplanes capillaceus* K95-55615 was used as an outgroup sequence

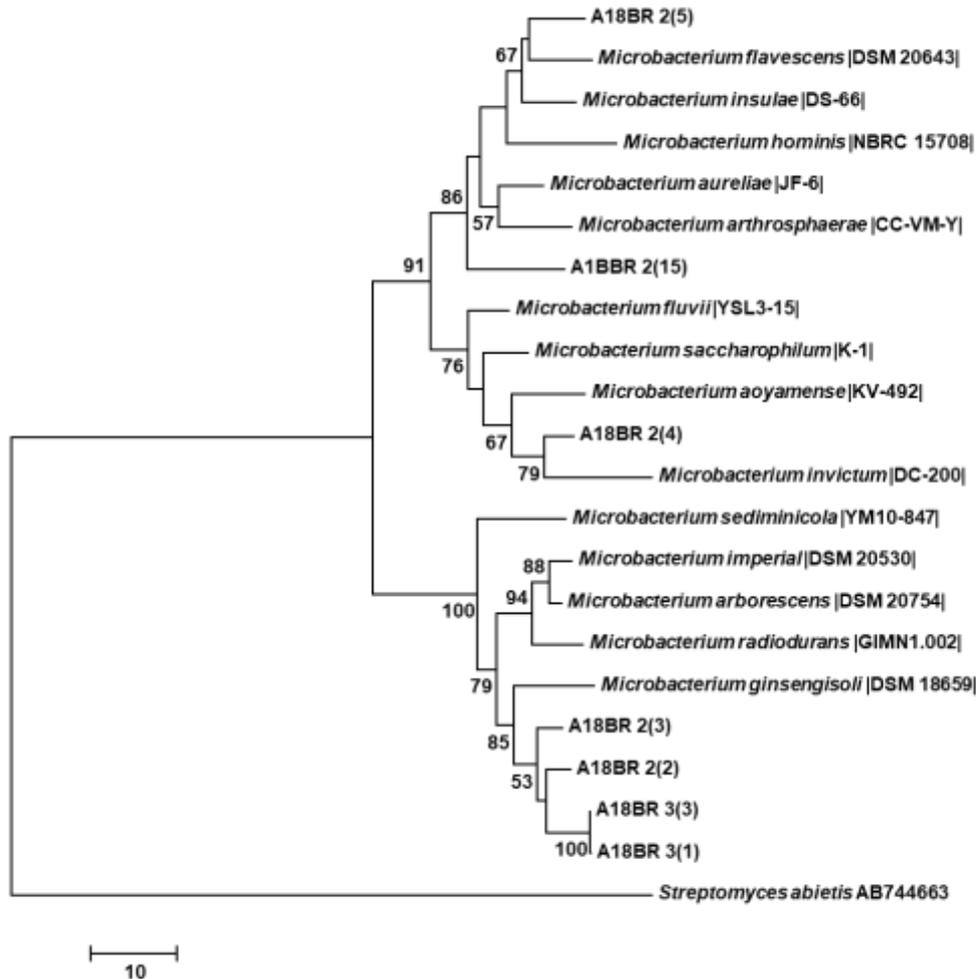


Figure 5. Neighbour-joining tree based on 16S rRNA gene sequences of actinomycetes isolated from mosses on the surface of decayed stone of Borobudur Temple and their closely related type strain within the family *Microbacterium*. Bootstrap values are expressed as percentages 1000 replications. Bootstrap values >50 are shown at branch points. The bar represents ten substitutions per nucleotide position. *Streptomyces abietis* AB744663 was used as an outgroup sequence

Screening of Phosphate-solubilizing Actinobacteria

Out of 37 isolates, seventeen isolates (45.94%) showed abilities to release soluble phosphate. The positive result was indicated by the formation of clear zones around the colony of actinomycetes on the PVK medium (Figure 7). Phosphate was released in a range of 7-27.5mm (Table 4). Most of the isolates that have abilities to release soluble phosphate belong to genus *Streptomyces* (88.24%). Only two isolates belong to the Rare Actinomycetes Group, A18BR 1(3) and A18BR 2(15). Both isolates were isolated from the decayed stone from the base of Borobudur temple. The isolates had similarity with *Nocardia thailandica* and *Microbacterium aureliae* based on 16S rRNA gene sequencing (Table 5). The isolates of actinomycetes have potential in dissolving phosphate. The

formation of an inhibition zone proves that the actinomycetes isolate can dissolve inorganic phosphate in the form of tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) that is contained in the media. The formation of clear zones can also be caused by the production of organic acid by actinomycetes (Sharma, Sayyed, Trivedi, & Gobi, 2013). Hamdali et al. (2008) reported that total of 55 isolates of actinomycetes isolated from three different Moroccan phosphate mining centres were able to soluble rock phosphate in the synthetic minimum medium (Hamdali et al., 2008). The result indicated that a large number of actinomycetes were isolated from a decayed stone of Borobudur Temple potential in dissolving phosphate. The isolates may trigger the growth of mosses on Borobudur stone, and they may cause an increase in biodeterioration of Borobudur Temple stone.

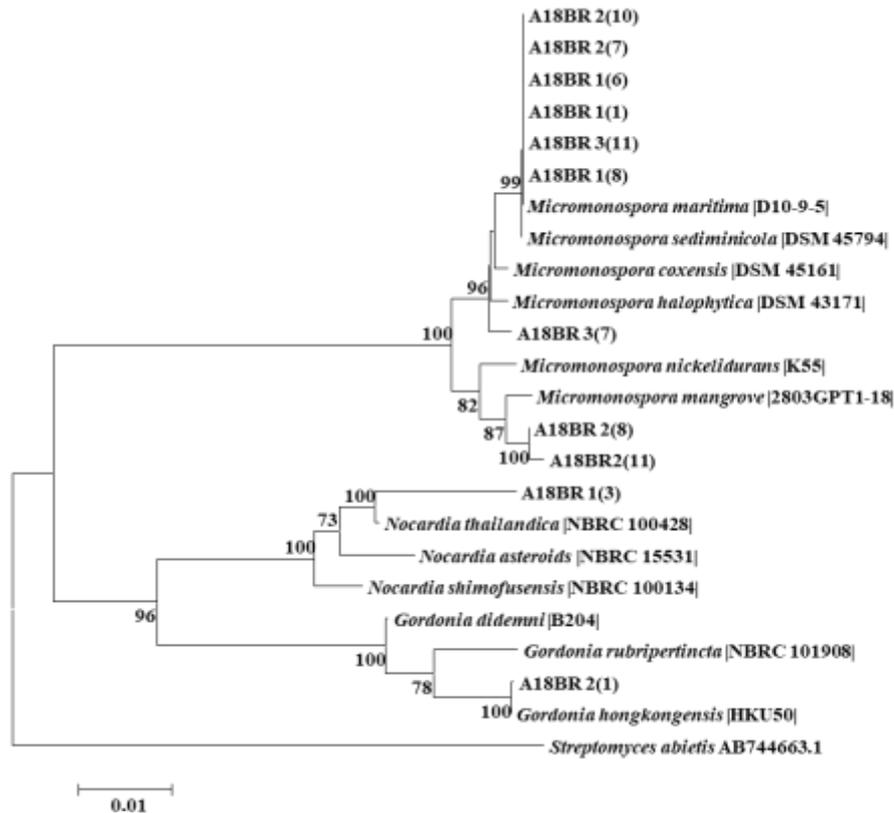


Figure 6. Neighbour-joining tree based on 16S rRNA gene sequences of actinomycetes isolated from mosses on the surface of decayed stone of Borobudur Temple and their closely related type strain within the family Micromonosporaceae and the family Nocardiaceae. Bootstrap values are expressed as percentages 1000 replications. Bootstrap values >50 are shown at branch points. The bar represents 0.01 substitutions per nucleotide position. *Streptomyces abietis* AB744663 was used as an outgroup sequence

Table 4. Phosphate solubilization actinomycetes isolated from moss on the decayed stone of Borobudur Temple

Isolates code	Species	Hallo zones index (mm)
A18BR1(2)	<i>Streptomyces chartreusis</i>	15
A18BR1(3)	<i>Nocardia thailandica</i>	12
A18BR1(10)	<i>Streptomyces griseoluteus</i>	11.5
A18BR2(9)	<i>Streptomyces polychromogenes</i>	14.5
A18BR2(12)	<i>Streptomyces avellaneus</i>	11
A18BR2(15)	<i>Microbacterium aureliae</i>	14
A18BR3(5)	<i>Streptomyces bungoensis</i>	20
A18BR3(6)	<i>Streptomyces griseoruber</i>	18
A18BR3(8)	<i>Streptomyces tritici</i>	9.5
A18BR3(9)	<i>Streptomyces bungoensis</i>	16
A18BR3(13)	<i>Streptomyces tritici</i>	10
A18BR3(14)	<i>Streptomyces griseoluteus</i>	18
A18BR3(15)	<i>Streptomyces neopeptinius</i>	15.5
A18BR3(17)	<i>Streptomyces violaceorectus</i>	10.7
A18BR3(19)	<i>Streptomyces echinatus</i>	27.5
A18BR3(20)	<i>Streptomyces polychromogenes</i>	7
A18BR3(21)	<i>Streptomyces wuyuanensis</i>	8

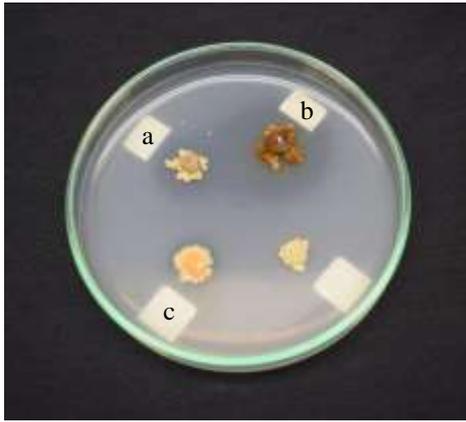


Figure 7. Zone of phosphate solubilisation around the colony growth of isolates a) A18BR 3(8), b) A18BR 3(5) and c) A18BR 3(20) on Pikovskaya's agar plate medium

The isolation and characterization of these actinomycetes from a decayed stone of Borobudur Temple is important not only to understand their role on the stone but also for taxonomical study of the actinomycetes from a decayed stone of Borobudur Temple. The isolates will be deposited to InaCC to enrich the collection of InaCC and to further research. Isolates showed different branched with others known of the species on the phylogenetic tree that taxonomic interest needs further study. On the other hand, the effect of the isolates triggers growth of mosses and their cause an increase in biodeterioration of Borobudur temple stones need further studied.

CONCLUSION

The environment condition and characteristic of stone of Borobudur Temple supported the present of actinomycetes at different tiers of Borobudur temple. The highest number of actinomycetes were isolated from decayed stones at the third tier (Stupa). The kind of medium used for isolation of actinomycetes may affect the number and variation of actinomycetes found. AIA medium support higher number and diversity of actinomycetes than HVA medium. The isolates of actinomycetes found belong to five genera (*Gordonia*, *Microbacterium*, *Micromonospora*, *Nocardia*, and *Streptomyces*). About 51.35 % of the actinomycetes isolates belong to genus *Streptomyces*. The actinomycetes isolates were capable of releasing soluble phosphate under laboratory test (45.94 %), and most of them were identified as *Streptomyces*.

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