Diversity of Actinomycetes from Soil Samples Collected from Lombok Island, Indonesia

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Abstract

A total of 137 strains of Actinomycetes were isolated from 14 soil samples collected in 3 part of Lombok Island, Indonesia using SDS-YE (SY) and Rehydration and Centrifugation (RC) isolation methods and Humic Acic Vitamin (HV) agar as isolation media. All the isolates were identified by morphological characteristic and by analysis of 16S rRNA gene sequence. On the basis of their morphology and 16S rRNA gene sequence, 67% of isolates were belonged to the Streptomyces Group and 33% of isolates were belonged to the Rare-Actinomycetes (Non-Streptomyces) Group. Nine families and 15 genera were found from the samples. It is indicated the richness of actinomycetes in these area. The genus *Streptomyces* is the most abundant in all soil samples, occupying 67% of all isolates by using SY method; while the genus *Actinoplanes* is mainly found by using RC as isolation method. Two strains belong to the genus *Virgisporangium* and *Catenulispora* that showed taxonomic interest need further study for describing as ne species.

Key words: Actinomycetes, Streptomyces Group, Non-Streptomyces Group, Diversity

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Introduction

Actinomycetes are a group of gram-positive bacteria that have high G+C contents. In general, actinomycetes could be divided into two group, (i) Streptomyces group which include only the genus *Streptomyces*, *Kitasatospora*, and *Streptacidiphillus*; and (ii) non-Streptomyces group or so-called rare actinomycetes which include the genus *Actinomadura, Kutsneria, Microbispora, Microtetraspora, Nonomuraea, Saccharomonospora, Streptosporangium, Thermobifida, Actinoplanes, Actinokineospora, Actinosynnema, Catenuloplanes, Cryptosporangium, Dactylosporangium, Geodermatophilus, Kineosporia* and *Sporichihya*.

Habitat of actinomycetes are common and ubiquitous in natural substrates, such as soils, where they usually play a significant role in the degradation of the more recalcitrant, naturally occurring organic polymers (Williams *et al*., 1984). Many actinomycetes are commercially important, either in the production of antibiotics and other bioactive secondary metabolites, or in useful biological processes (Okami & Hotta, 1988). Therefore, the isolation and subsequent characterization of these organisms from diverse habitats is important not only to understand their role in natural ecosystems but is also of value for establishing novel strains with pharmaceutical and industrial applications.

Since the discovery of the antibiotic actinomycin in 1940 and streptomycin in 1944 by Waksman and co workers, much attention has been focused on the genus *Streptomyces*, the most abundant and recoverable actinomycete occurring in soil. Currently, the number of species of this genus is more than 400 species. Although the genus *Streptomyces* still continue to be a major source of isolation to search bioactive compounds, the rate of discovery of new metabolites from these ubiquitous species has declined.

In order to find less-known or new taxa of actinomycetes as well as to reduce the re-

isolation of strains producing known bioactive compounds, the adoption of reliable methodologies for isolating actinomycetes have been developed and applied by using the information generated through microbial physiology and ecology (Goodfellow & Williams, 1986; Okami & Hotta 1988; Bull *et al*., 2000). Selective isolation methods to recover rare actinomycetes are then to be the major targets in the search for novel antibiotics from actinomycetes (Lazzarini *et al*., 2000).

Additionally, to obtain new strains likely to produce novel metabolites, examination of samples from diverse habitats as well as the ones inhabiting at unexplored yet environments is also necessary. It is undoubted that the selection of novel bioactive producing microorganisms from nature requires a sound microbial taxonomical knowledge and fuller understanding of microbial ecology and physiology as means for revealing novelty (Goodfellow & Williams, 1986).

During the joint collaboration research between Indonesia and Japan on Taxonomical and Evaluation Study of Actinomycetes from Indonesia, we isolated actinomycetes from Lombok Island. This is the aim of this study to identify and study the diversity actinomycetes from Lombok Island soil samples by selective isolation methods.

Materials and Methods

Materials, sampling sites, and samples treatment. Fourteen soils collected from 3 locations in Lombok Island, Indonesia (Table 1) were used as samples. Seven samples were collected from West Lombok around UPT Loka Pengembangan Bio Industri Laut Mataram, LIPI (coordinate: 8°24'23-46"S 116°4'52-59"E, altitude: 73-177, temperature: 30-31°C, and humidity: 50%), 5 samples were collected in East Lombok in mangrove forest (coordinate: 8°54'31-49"S 116°20'28-43"E, altitude: 0-20, temperature: 29°C, and humidity: 62-66%), and 2 samples were collected from Center Lombok in completely dried paddy fields (coordinate: 8°45'59-60"S 116°15'49-53"E, altitude: 110, temperature: 28-30°C, and humidity: 56-63%). The samples were air dried for at least 7 days, were ground with a blender and sieved, then 1 g of samples were used for isolation of actinomycetes.

Isolation method. SDS-Yeast Extract (SY) isolation method and Rehydration-Centrifugation (RC) isolation method (Hayakawa *et al.*, 2000) were employed to isolate actinomycetes. Humic acid-vitamin (HV) agar supplemented with nalidixic acid and cycloheximide (Hayakawa & Nonomura, 1987) were used as isolation medium. Yeast Extract and Starch (YS) agar were used for maintaining of isolates. All plates were incubated at 30°C for 2-3 weeks.

Morphological characterization. Actinomycetes were examined by eye and by using a light microscope and tentatively identified up to group rank based on morphological criteria.

16S rRNA gene sequencing. Genomic DNA was extracted as described by Saito & Miura (1963) and 16S rRNA genes was amplified using TaKaRa Taq polymerase (Takara Shuzo, Kyoto, Japan) and the primer pairs, 9F (5`-GAGTTTGATCCTGGCTCAG) and 1541R (5`-AAGGAGGTGATCCAGCC). Amplified 16S rRNA gene (1.5 kb) was purified and directly sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif., USA) as specified by the manufacturer. The sequencing primers were: 9F (5`-GAGTTTGATCCTGGCTCAG), 515F (5`-GTGCCAGCAGCCGCCGCGGT), 536R (5`-GTATTACCGCGGCTGCTG), 785F (5`-GGATTAGATACCCTGGTAGTC), 802R (5`-TACCAGGGTATCTAATCC), 1099F (5`-GCAACGAGCGCAACCC), 1115R (5`-AGGGTTGCGCTCGTTG), and 1541R (5`-AAGGAGGTGATCCAGCC). An Applied Biosystems PRISM 310 Genetic Analyser performed electrophoresis of the sequencing reaction mixtures. The 16S rRNA gene sequences determined in this study were manually aligned with the published sequences of reference strains available from the EMBL/GenBank/DDBJ databases (Altschul *et al*., 1998).

Construction of phylogenetic tree. The CLUSTAL W software package (Thompson *et al*., 1994) generated evolutionary distances (the K_{nuc} value of Kimura (1980)) and similarity values. A phylogenetic tree was constructed by neighbour-joining (Saitou & Nei, 1987) from K_{nuc} values. The topology of

the phylogenetic tree was evaluated by bootstrap re-sampling as described by Felsenstein (1985) with 1000 replicates.

Results and Discussion

The number and identification of isolates

A total of 137 actinomycetes isolated from 14 soils collected from 3 locations in Lombok Island were selected in 2006 (Table 1). The selected isolates by using SY as isolation method was 98 isolates and by using RC was 39 isolates. SY and RC isolation methods were usually employed to isolate rare actinomycetes and further the Rehydration-Centrifugation (RC) method especially was employed to isolate motile zoospores bearing rare actinomycetes. But using two methods, Streptomyces Group also still could be isolated from environmental samples, due to spore of Streptomyces Group is abundant in soils.

All the selected isolates were identified by sequencing of 16S rRNA gene. On the basis of their morphology, 92 isolates (67%) were belonged to the Streptomyces Group and 45 isolates (33%) were belonged to the Rare-Actinomycetes (Non-Streptomyces) Group. The morphological differences of Streptomyces and Rare-actinomycetes Group are as follows; Streptomyces Group is fast growth actinomycetes, with aerial mycelium, and usually do not has motile of zoospore. Non-Streptomyces Group (Rare Actinomycetes) has small colony, slow growth, usually no aerial mycelium, and has motile of zoospore. Further, analysis of 16S rRNA gene sequencing identified the isolates into genus or species level.

Table 1. The number of isolates from soil samples

Sampling Site	Sampling Source	Number of samples	Isolation Method*	Selected isolates	
Lombok Island	Soils under tropical plants in Gili area	7	SY	54	80
			RC	26	
	Soils under mangrove in Kuta area	5	SY	29	33
			RC	4	
	Soils in rice fields in center area	2	SY	15	24
			RC	9	
Total		**14**		**137**	**137**

*SY: SDS-Yeast Extract Isolation Method, RC: Rehydration Centrifugation Isolation Method

Diversity of Actinomycetes

The diversity of actinomycetes at the genus level is shown in Table 2. On the basis of 16S rRNA gene analysis, 15 genera of Actinomycetes were isolated from 14 soil samples collected from Lombok Island. From 7 soil samples collected in West Lombok area, 13 genera of actinomycetes were found; from 5 soil samples collected in East Lombok (mangrove) areas, 3 genera were found; while from 2 soil samples collected from rice paddy field, 5 genera were found. The genus of *Streptomyces* and *Micromonospora* were found in all three areas. Eighty isolates obtained from soils in West Lombok areas were identified as *Dermacoccus*, *Agrococcus*, *Actinoplanes*, *Couchioplanes*, *Dactylosporangium*, *Virgisporagium*, *Nocardia*, *Kribbella*, *Nocardioides*, *Pseudonocardia*, *Streptomyces*, *Nonomuraea*, and *Microbispora*. The genera *Agrococcus*, *Actinoplanes*, *Couchioplanes*, *Dactylosporangium*, and *Microbispora* were mainly isolated by RC method and *Dermacoccus*, *Virgisporagium*, *Kribbella*, *Nocardioides*, and *Streptomyces* were isolated by SY method. *Streptomyces* was predominant in this area when isolated using SY method and *Actinoplanes* was predominant when isolated using RC method (Hayakawa, 2008). This report confirmed many study on isolation of actinomycetes reviewed by Goodfellow and William (1983) that the predominant actinomycetes in soil samples are the genus *Streptomyces*.

Actinomycetes isolated from soils/sediment in mangrove areas have been studied by Niladevi & Prema (2005) and Sivakumar *et al*. (2005). They found that several *Streptomyces* in mangrove sediment and water are useful for enzymes production. As mentioned by previous reports, in this study, from 5 soil samples of mangrove sediment, we obtained 33 isolates of actinomycetes and the isolates belonged to 3 genera, *Catenulispora*, *Micromonospora*, *Streptomyces*. *Streptomyces* was predominant in mangrove sediments.

Rice paddy field also a good source of actinomycetes. Suzuki *et al*., (1994) reported that Streptomycetes, Micromonosporaceae, Nocardioform was abundant in rice paddy field collected from two areas in Japan. In this study, we only collected two soil samples of paddy field with the condition completely dried. From only 2 samples, we isolated and selected 24 actinomycetes, and among them *Streptomyces* was predominant by using both 2 isolation methods.

Table 2. Diversity of actinomycetes by sources and isolation methods

Sample No.	Family Name		Genus Name	Isolation Method		Total
				SY	RC	
Soils under tropical plants (80 isolates)	*Dermacoccaceae*	1	*Dermacoccus*	1		**1**
	Microbacteriaceae	2	*Agrococcus*		1	**1**
	Micromonosporaceae	3	*Actinoplanes*	1	11	**12**
		4	*Couchioplanes*		1	**1**
		5	*Dactylosporangium*		1	**1**
		6	*Virgisporangium*	1		**1**
	Nocardiacea	7	*Nocardia*	2	1	**3**
	Nocardioidaceae	8	*Kribbella*	1		**1**
		9	*Nocardioides*	1		**1**
	Pseudonocardiaceae	10	*Pseudonocardia*	3	1	**4**
	Streptomycetaceae	11	*Streptomyces*	42	6	**48**
	Streptosporangiaceae	12	*Nonomuraea*	3	2	**5**
		13	*Microbispora*		1	**1**
Soils under mangrove (33 isolates)	*Catenulisporaceae*	1	*Catenulispora*	1		**1**
	Micromonosporaceae	2	*Micromonospora*	6		**6**
	Streptomycetaceae	3	*Streptomyces*	22	4	**26**
Soils in rice fields (24 isolates)	*Dermacoccaceae*	1	*Dermacoccus*	2		**2**
	Micromonosporaceae	2	*Actinoplanes*	2		**2**
		3	*Micromonospora*	1		**1**
		4	*Pseudonocardia*	1		**1**
	Streptomycetaceae	5	*Streptomyces*	11	7	**18**
Total				**101**	**36**	**137**

Phylogenetic tree of Genus *Virgisporangium*

The genus *Virgisporangium* was firstly described by Tamura et al (2001) for actinomycetes that are characterized by motile spores, and containes three species, namely *Virgisporangium auranticum*, *Virgisporangium ochraceum*, and *Virgisporangium aliadipatigenens* (Otoguro *et al*., 2010), all of them isolated in Japan. One isolate, ID06-A0187 was affiliated within the cluster of the genus *Virgisporangium* (Figure 1) and showed different branch with other known species of this genus. This is the first *Virgisporangium* found in Indonesia.

Phylogenetic tree of Genus *Catenulispora*

The genus *Catenulispora* has been well characterized based on acidophilic and currently contains five species, namely *Catenulispora acidophila* (Busti *et al*., 2006), *Catenulispora rubra* (Tamura *et al*., 2007), *Catenulispora subtropica* (Tamura *et al*.,

2008), *Catenulispora yoronensis* (Tamura *et al*., 2008) and *Catenulispora graminis* (Lee *et al*., 2011). In this study, we isolated one *Catenulispora* strain, ID06-A213, that affiliated in the clustered of the genus *Catenulispora* based on 16S rRNA gene sequencing, but showed independent branch with other species (Figure 2). Further, taxonomical study of the isolate compare with known species is needed.

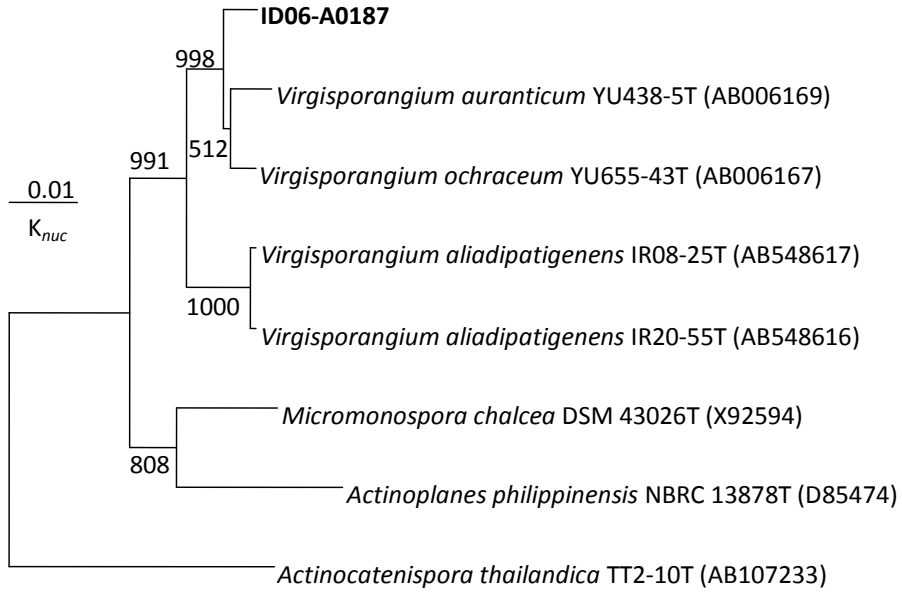

Figure 1. Phylogenetic tree based on 16S rRNA gene sequencing of ID06-A0817 to the family *Micromonosporaceae*. The tree was constructed using the neighbor joining method. Bar, 0.01 K_{nuc}.

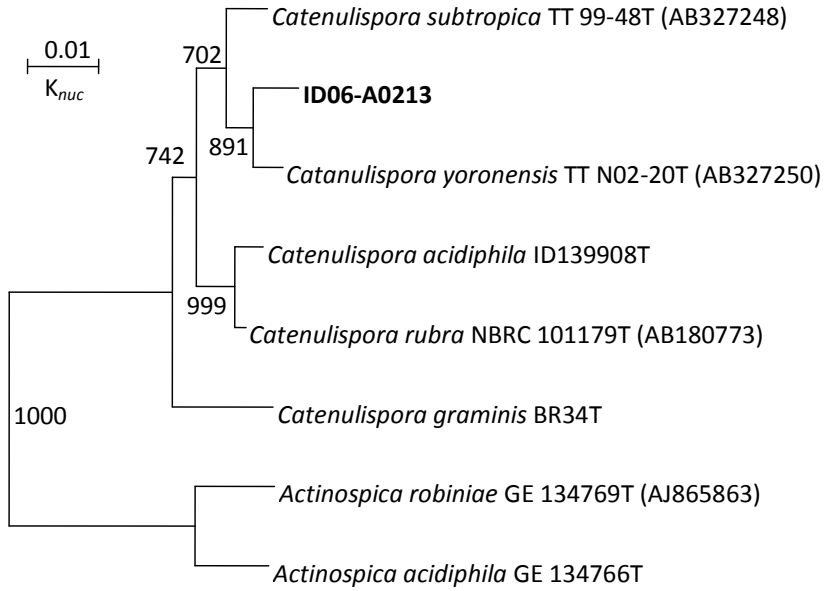

Figure 2. Phylogenetic tree based on 16S rRNA gene sequencing of ID06-A0213 to the family *Catenulisporaceae*. The tree was constructed using the neighbor joining method. Bar, 0.01 K_{nuc}.

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