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Submission date: 30-Jan-2020 04:20PM (UTC+0800)
Submission ID: 1248661331
File name: Identification_of_Streptomyces....pdf (106.21K)
Word count: 804
Character count: 4627

IDENTIFICATION of *Streptomyces* sp. SOIL ISOLATE SIDOARJO LAPINDO MUD BASED on GENE 16S rRNA

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Abstract

This study was aimed to identify the Streptomyces sp. soil isolates of Lapindo Sidoarjo mud based on 16S rRNA gene. Several stages of research was conducted to identify Streptomyces sp. isolates of Lapindo Sidoarjo mud based on 16S rRNA gene. The isolation of Streptomyces sp. from Lapindo Sidoarjo mud was conducted by several stages. The process was divided into isolation, purification and identification of Streptomyces sp. isolates based on its morphology. The next process was DNA isolation, purification, and identification of Streptomyces sp. isolates of lumpur Lapindo Sidoarjo mud based on 16S rRNA gene. The isolation process resulted eight isolates of Streptomyces sp. with morphologically distinct characters. The DNA isolation and identification based on 16S rRNA gene showed 8 isolates of Streptomyces sp. of lumpur Lapindo mud has ribbon at 1500 bp long.

Keywords : Streptomyces sp., Lapindo Sidoarjo mud, 16S rRNA gene

Introduction

Actinomycetes are a group of bacteria that have a high biodiversity and the potential to generate novel species. Actinomycetes have a morphology similar to fungi. In common with fungi lies in the structure of filament-shaped soft actinomycetes (hyfa / mycelia) (Rao, 2001). At this time a lot of research that is focused on actinomycetes, particularly Streptomyces which is indicated as bacteria capable of generating most antibiotics. Streptomyces have an important role in the field of biotechnology, because it can produce some bioactive secondary metabolites are antibiotics. Habitat actinomycetes, particularly Streptomyces is on the ground, including the Lapindo Sidoarjo mud. Approximately 70% of the microbes in the soil is Streptomyces (Kyuma, 2000). Until now, many Streptomyces genome as a source of biodiversity has not been studied, so research 16S rRNA gene sequences of Streptomyces sp. of isolates Lapindo Sidoarjo mud needs to be done.

Material and Methods

This research is the laboratory exploration. Several steps were taken as follows :

1). Isolation of Streptomyces from mud Lapindo done by culturing suspension of mud on ISP medium-4 Agar. Purification is done by culturing repeatedly until a colony of Streptomyces in accordance with morphological features.

2). Identification of the isolates by 16S rRNA gene carried by DNA isolation using a Qiagen kit. Furthermore, the PCR using (Davelos *et al.* 2004):

foward pA 5'-AGAGTTTGATCCTGGCTCAG-3'

reverse pH 5'AAGGAGGTGATCCAGCCGCA-3'

Amplification was performed for 35 cycles: denaturation 95 ° C for 1 min, annealing 60 ° C for 1 min, elongation 72 ° C for 1 minute. In the 35th round of post elongation of 72 ° C for 10 minutes.

The next step is electrophoresis of PCR products using 2% agarose gel.

Result and Discussion

Isolated mud Lapindo Sidoarjo obtained eight isolates showing morphological characteristics of Streptomyces. Streptomyces identification based on morphological visible colonies with slippery surfaces and form mycelium after incubation few days with various colors include yellow, pink and gray with white spores. The big difference in the color of the isolates of Streptomyces made possible because of the influence of age colony, carbon or nitrogen source (Oskay, 2011).

Identification of Streptomyces by 16S rRNA gene carried as confirmation that isolates the Streptomyces isolates. Results PCR isolates suspected of Streptomyces sp. Figure 1 shows that the eight isolates Streptomyces produce one dominant band measuring approximately 1500 bp corresponding to the size of the 16S rRNA gene.

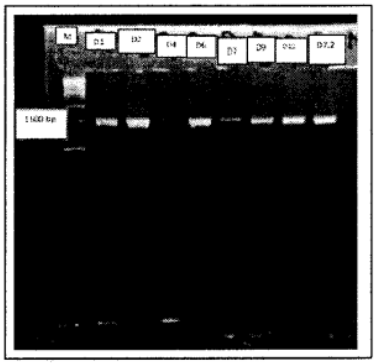

Figure 1. PCR Product of Lapindomud isolates

Identification of the bacteria can be done using 16S rRNA gene by the method of Polymerase Chain Reaction (PCR). Identification of Streptomyces sp. can use the 16S rRNA gene for 16S RNA sequences 1500 bp in length is a conservative area (not much change from one organism into another organism) so that the sequence obtained is used for microbial identification (Clarridge, 2004).

Conclusion

The DNA isolation and identification based on 16S rRNA gene showed 8 isolates of Streptomyces sp. of lumpur Lapindo mud has ribbon at 1500 bp long.

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