Diversity of DNA polymerase I gene fragments from domas hot spring

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Abstract

DNA polymerase is an enzyme that has a main role in molecular biotechnological techniques. It is used in PCR technique, DNA labeling and DNA sequencing. Most of thermostable DNA polymerases are expressed by thermophilic and hyperthermophilic organisms, isolated from geothermal, hot spring and other high temperature area. Indonesia has many places that are suitable as the habitat of thermophilic and hyperthermophilic microbes; however these microbes are not easy to be cultivated. In order to clone the DNA polymerase I gene from these microorganisms, we use metagenomic approach to amplify the gene directly from natural sample. Total communities DNA were extracted from water sample of Domas hot spring, West Java, by using modified Zhou method. PCR amplification was performed with a set of internal primer targeting DNA polymerase I gene fragment with additional 40 GC clamp oligomer at reverse primer. This set of primers amplify approximately 600 bp fragment of thermostable DNA polymerase I gene. The amplicon from PCR then analyzed through Denaturing Gradient Gel Electrophoresis (DGGE) to separate the fragments, based on the GC content composition. DGGE analysis showed that the fragment was separated in many bands. This is suggested that the amplicons contain many variants of thermostable DNA polymerase I genes.

Keyword: thermostable DNA polymerase, metagenomic approach, Domas hot spring, DGGE analysis

Introduction

DNA polymerase is an enzyme that essential for all living organisms. It has a main role in the replication and maintenance of the genome; it is a central to the accurate transmission of generation to the next that are present in living cells. The DNA polymerases nomenclature was suggested based on the similarity of their sequences, now DNA polymerases can be classified into A, B, C, D, X, Y and RT families (Patel et al. 2001). DNA polymerase I was classified as Pol I family or Pol A family that is similar to E. coli Pol I or human Pol A.

Thermostable DNA polymerases are used extensively in biotechnological techniques, especially in molecular biological techniques as PCR technique, DNA labeling and sequencing. Most of thermostable DNA polymerases are expressed by thermophilic and hyperthermophilic organisms, which live in geothermal, hot spring and other high temperature area. Indonesia is one of the countries that located in the Pacific Ring of Fire, it caused that Indonesia has many geothermal, hot springs and crater which are suitable as the habitat of thermophilic and hyperthermophilic microbes. Recent researches obtain that various thermophilic and hyperthermophilic microbes were live in many hot springs at West Java. However, these microbes were not easy to be cultivated. In order to clone the DNA polymerase gene from these microorganisms, we use metagenomic approach to amplify the gene directly from natural sample.

In this report we described the diversity of DNA polymerase I gene fragments from Domas Hot Spring.

Materials and Methods

Materials

The microbial sample was collected from Domas Hot Spring, field of Tangkuban Perahu volcano, West Java. This hot spring has temperature around 92°C to 95°C and pH around 1 to 3 respectively.

The primers for PCR amplification are:

FP1: GATCCGAACCTGCAAAACATTCC
RP1GC: CGCCCGCCGCGCGCGGCGGGGCGGGGGAGAACGGCTCGTCATGGACC

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