Isolation and identification of thermostable \(\alpha\)-amylase producing thermophilic bacteria from Cangar-Batu hot spring

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Abstract

Thermostable enzyme is produced by thermophilic organisms that live in a relatively high temperature such as in hot springs of Cangar Batu Malang. This research has been done to isolate and identify thermophilic and amylolitic bacteria to know the types and activities. This study consists of five phases: (1) sampling at hot springs Cangar-Batu, (2) isolation of thermophilic and amylolitic bacteria, (3) morphological identification of thermophilic and amylolitic bacteria, (4) qualitative and quantitative analysis of thermostable amylase activity, (5) identification of thermophilic amylolitic bacteria. Qualitative amylase activity has been seen by the clear zone formed in iodine selective media, while quantitative activity assay performed by measuring the glucose levels of starch degradation using the Somogyi-Nelson method. The study has shown that from a hot spring Cangar-Batu has been isolated thermophilic and amylolitic bacteria, that grown at 55°C incubation temperature, the clear zone of amylolitic is 20 mm in diameter, whereas the activity of crude enzyme extract is 2.175 units/mL. Species identification results showed that the bacterium is \textit{Bacillus alvei} (Paenibacillus alvei). Identification based on 16S RNA gene analysis is being performed.

Keywords: thermophilic bacteria, thermostable amylase, Cangar-Batu

Introduction

Thermophilic microorganisms capable of synthesizing heatresistant molecules, including enzymes. The application of thermostable enzymes on biotechnological processes can reduce operational costs and increase the reaction rates thus increase the productivity (Aquilar \textit{et al.}, 1998). This relates to the benefits to that can be obtained when the production process carried out at high temperatures, to reduce contamination, increase the mass transfer rate and reduce the viscosity of the solution.

For industrial applications, enzymes must be stable under process conditions. Thermophilic microorganisms are believed to be potentially sources of thermostable enzymes (Egas \textit{et al.}, 1998). Hotsprings are a good source for the isolation of thermophiles. Thermostable enzymes have been reported to have higher stability to organic solvents, acidic and alkaline pH and detergents (Vieille \textit{et al.}, 1996). One of the enzymes that widely produced and used is amylase.

Amylase has an important role in industrial and biotechnology applications in food, textiles, and paper industries (Pandey \textit{et al.}, 2000). Thermostable \(\alpha\)-amylase has had commercial application in the process of starch, food, and sugar production (Leveque \textit{et al.}, 2000), the textile industry (Pedersen \textit{et al.}, 1999), and detergent (Hewitt & Solomons, 1996; Lin \textit{et al.}, 1998). Industrial applications required \(\alpha\)-amylase that can be produced in large quantities and high activity with economical production costs. Indonesia is expected to meet the need for enzymes in the country itself, so it can lower the production cost. Thermophilic amylase preferred for practical applications since the hydrolysis of starch is known to accelerate the reaction rate at high temperatures and produce a lot of important products with different chemical and physical properties for food and industry.

Amylase which has a thermostable properties can be obtained from thermophilic microorganisms (Leveque \textit{et al.}, 2000). The existence of the microorganisms is one important factor in enzyme production business. Therefore, the searches for thermostable amylase-producing microorganisms need to be done in Indonesia. High biodiversity in Indonesia provides a great opportunity to get the thermophilic microorganisms with the potential to be developed as a enzymes producer.

The purpose of this study is to isolate bacteria from hot springs thermophilic Cangar-Batu, screening isolates that produce \(\alpha\)-amylase enzyme, and identify the type of thermophilic amylolitic bacteria.

Materials and Methods

Materials

Materials used in this study are the hot water samples taken from the location of Cangar-Batu hot springs, medium NA (Nutrient agar), Media NB