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Original research article

# Isolation and Phylogenetic Analysis of Thermophile Community Within Tanjung Sakti Hot Spring, South Sumatera, Indonesia

Heni Yohandini,[1*] Julinar,[1] Muharni[2]

[1] Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Sriwijaya, Ogan Ilir 30662, Indonesia.
[2] Department of Biology, Faculty of Mathematic and Natural Sciences, Universitas Sriwijaya, Ogan Ilir 30662, Indonesia.

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ABSTRACT

A community of thermophiles within Tanjung Sakti Hot Spring (South Sumatera) have been cultivated and identified based on 16S ribosomal RNA gene sequence. The hot spring has temperature 80 °C–91 °C and pH 7–8. We used a simple method for culturing the microbes, by enriching the spring water with nutrient broth media. Phylogenetic analysis showed that the method could recover microbes, which clustered within four distinct taxonomic groups: *Anoxybacillus*, *Geobacillus*, *Brevibacillus*, and *Bacillus*. These microbes closely related to *Anoxybacillus rupiensis*, *Anoxybacillus flavithermus*, *Geobacillus pallidus*, *Brevibacillus thermoruber*, *Bacillus licheniformis*, and *Bacillus thermoamylovorans*. The 16S ribosomal RNA gene sequence of one isolate only had 96% similarity with *Brevibacillus* sequence in GenBank.


## 1. Introduction

Hot springs are unique areas that are characterized by high temperature and have a great diversity of natural environments. These areas are habitats for a diversity of thermophile, particularly belonging to bacteria and archaea domains. Thermophile diversity provides an overview of the enormous potential that can be utilized for various purposes. Thermophilic microbes are currently studied intensively for reasons of development of basic research and biotechnological applications. In addition, a study of thermophile led to the discovery of novel species (Huber *et al.* 1991; Kozina *et al.* 2010; Zhang *et al.* 2010).

Microbial diversity in a community can be studied through culture-dependent and culture-independent approaches. Researchers believe that the traditional approach which depends on cultivation for identification has many limitations to discover a broad diversity of microbial communities in their natural habitats. However, pure cultures were required for characterization in understanding microbial physiology and genetics (Ward *et al.* 1998). Indonesia has over 70 active volcanoes and a large number of geothermal regions and abundant hot springs (Kusumadinata 1979). The diverse terrestrial hydrothermal regions have potential habitat for large communities of thermophilic microbes. To date, information about microbial diversity from Indonesian hot spring is very limited. Studies on Indonesian thermophile communities have been conducted in some geothermal area residing in the Javanese island (Aditiawati *et al.* 2009; Aminin *et al.* 2008; Baker *et al.* 2001; Huber *et al.* 1991; Lohr *et al.* 2006; Yohandini *et al.* 2008). These studies have discovered high diversity of thermophilic/hyperthermophilic bacteria and archaea from Indonesian thermal area. In this report, we describe the first diversity study of culturing thermophile isolated from Tanjung Sakti Hot Spring, located in South Sumatera. We used simple enrichment method to cultivate the microbes and analysis of its 16S ribosomal (rRNA) gene sequences for microbial idenfication. Microbial isolates obtained in this study were also intended to complement the microbial culture collection that can be used as a source of thermostable enzymes and secondary metabolites, besides for the purposes of bioremediation.

## 2. Materials and Methods

### 2.1. Materials

Spring water was taken from Tanjung Sakti Hot Spring on April 17, 2013. Nutient broth and nutrient agar media (Oxoid), GoTaq Green Master Mix, DNA Purification Kit (Promega), 27F primer: AGAGTTTGATCATGGCTCAG and 1492R primer: GGGTACCTTGTTACGACTT (Macrogen), Agarose (Sigma), chloroform, ethanol, isoamylalcohol, and isopropanol (Merck).

* Corresponding author.
*E-mail address:* heniyo@yahoo.com (H. Yohandini).
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Table 1. The highest homology of 16S ribosomal RNA gene sequence of Tanjung Sakti isolates with existing data in GenBank

| Isolate code | Taxonomic affinity | % Homology |
|---|---|---|
| TS-01, TS-04 | *Anoxybacillus* sp. | 99% |
| | *Anoxybacillus rupiensis* | 99% |
| | *Anoxybacillus beppuensis* | 99% |
| | *Anoxybacillus amylolyticus* | 99% |
| TS-03, TS-08, TS-09, TS-10, TS-11, TS-16, TS-17 | *Bacillus* sp. | 99% |
| | *Bacillus licheniformis* | 99% |
| TS-05, TS-06 | *Brevibacillus* sp. | 100% |
| | *Brevibacillus thermoruber* | 100% |
| TS-07 | *Brevibacillus* sp. | 96% |
| | *Brevibacillus thermoruber* | 96% |
| TS-12 | *Geobacillus* sp. | 99% |
| | *Geobacillus pallidus* | 99% |
| TS-15 | *Anoxybacillus* sp. | 99% |
| | *Anoxybacillus flavithermus* | 99% |
| TS-18 | *Bacillus* sp. | 99% |
| | *Bacillus thermoamylovorans* | 99% |
| | *Bacillus circulans* | 99% |

## 2.2. Sampling site and cultivation

Microbial samples were taken from the Tanjung Sakti Hot Spring, located in Lahat Regency, South Sumatra. Approximately 25 mL of spring water was added to 1 mL of sterile nutrient broth (NB) medium. Samples were kept at high temperature during the trip back to the laboratory. Subsequently, samples were incubated at aerobic condition with 55 °C. Pour plate method was used to obtain pure cultures. Isolates were separated based on differences in the shape, color, and size of the colony.

## 2.3. DNA extraction and 16S rRNA gene amplification

Chromosomal DNA was extracted using the DNA Purification Kit (Promega). Microbial pellet cells were suspended and incubated at room temperature in cell lysis solution for 10 minutes, added with nuclei lysis solution containing RNase and incubated at 37 °C for 15 minutes, and then added with protein precipitation solution, and centrifuged at 13,000 rpm for 10 minutes at 20 °C. The DNA was purified three times with equal volumes of chloroform:isoamylalcohol (24:1), precipitated with 0.6 volumes of isopropanol, washed in 70% ethanol, and resuspended in dd$H_2O$. 16S rRNA gene fragment was amplified by polymerase chain reaction using primers 27F and 1492R (Baker *et al.* 2003; Frank *et al.* 2008). Master mix reaction, including *Taq* DNA polymerase enzyme (GoTaq Green Master Mix, Promega) was used according to standard usage. The reactions were incubated in a C-1000 Thermal Cycler (Bio-Rad, USA) for 3 minutes at 95 °C, and then for 30 cycles of 30 seconds at 95 °C, 1 minute at 55 °C, and 1 minute and 30 seconds at 72 °C, and a final extension of 5 minutes at 72 °C. All the 16S rRNAs gene fragments were sequenced using commercial services at Macrogen Inc., Korea. All sequences were submitted to GenBank and assigned by KJ842627 to KJ842641.

## 2.4. Phylogenetic analysis

Nucleotide sequences were compared for homology with BLAST search (http://www.ncbi.nlm.nih.gov) (Altschul *et al.* 1990).

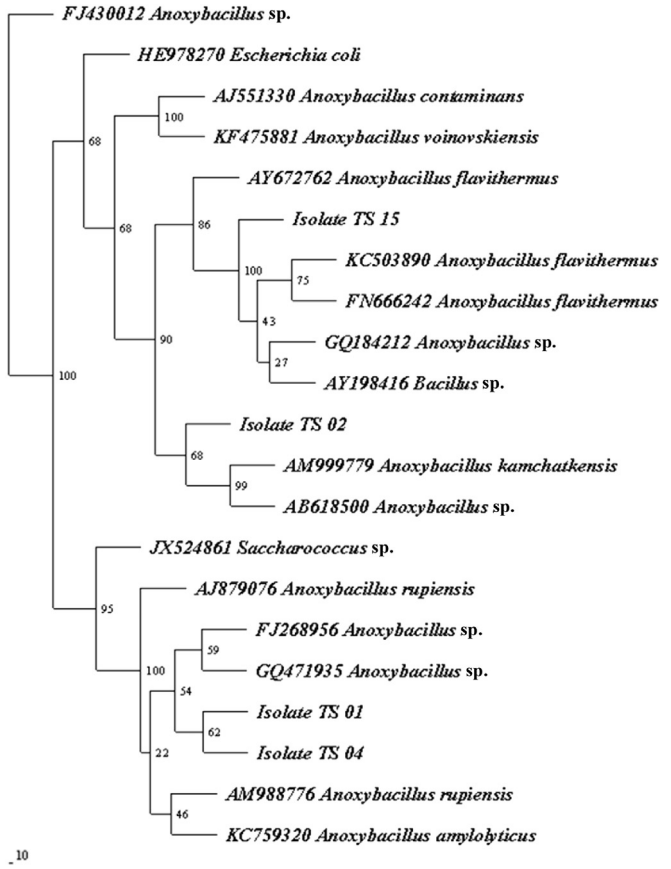


**Figure 1.** Phylogenetic tree of Tanjung Sakti isolates which have the highest homology with *Anoxybacillus*. The phylogenetic was constructed using neighbor joining method with 100 bootstrap replicates.

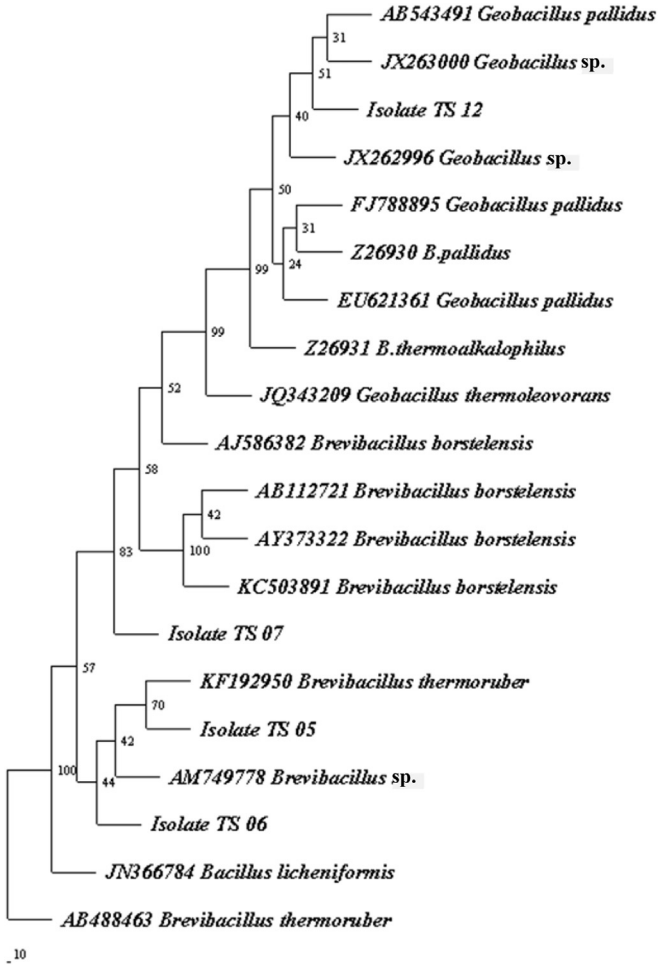


**Figure 2.** Phylogenetic tree of Tanjung Sakti isolates which have the highest homology with *Brevibacillus* and *Geobacillus*, constructed using neighbor joining method with 100 bootstrap replicates.