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Molecular Identification Using 1S6 rRNA Gene of Amylase Producing Bacteria (S2.3 Isolate)

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ABSTRACT

Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze starch to simple sugar. Amylase is one of the three largest industrial groups of enzymes and accounts for approximately 65% of the world's enzymes. Amylase produced by thermophilic bacteria is commonly found in volcanic craters, craters on the ocean floor, composted soil, and hot springs. Pariangan hot spring, West Sumatra, Indonesia has a high level of bacterial diversity. The methods used in this research are genomic DNA isolation, DNA isolation, and analysis of the 16S rRNA gene. Bacterial screening results obtained S2.3 bacterial isolates with the highest activity but the type of bacterial isolates S2.3 is unknown. The purpose of this study was to determine the type of bacterial isolate S2.3 originating from the Pariangan hot spring, West Sumatra, Indonesia using 16S rRNA gene analysis. The research methods are genomic DNA isolation**,** DNA amplification, and 16S rRNA gene analysis. The results showed that the bacterial isolate S2.3 had a 90% similarity with the *Bacillus licheniformis* strain ATCC 14580.

1. INTRODUCTION

Bacteria can live and thrive in extreme habitats which range from 45 ºC to 80 ºC. Thermophilic bacteria can produce a variety of enzymes for biotechnology and industry, such as proteases, lipases, cellulases, and amylase. Amylase produced by bacteria or bacteria amylase is an enzyme that can hydrolyze simple starch to sugar [1]. Amylase produced by thermophilic bacteria is not denatured, active, and stable at high temperatures [2]. Amylase is one of the three largest industrial groups of enzymes and accounts for approximately 65% of the world's enzymes [3]. In the field of the food industry, amylase plays a role in the manufacture of glucose syrup, bread making, and baby food. In the field of the non-food industry, amylase plays a role on the paper industry, leather tanning, pharmaceuticals, textiles, and etergent additive [4].

Amylase produced by thermophilic bacteria is commonly found in volcanic craters, craters on the ocean floor, composted soil, and hot springs. Indonesia is rich in hot springs and generally, water sources have a pH of 7. Hot springs in Pariangan, West Sumatra, Indonesia has a temperature of 47 ºC - 51 °C and a pH of 9.2 or is alkaline. Pariangan hot spring, West Sumatra, Indonesia has a high level of bacterial diversity. Isolation and screening of amylase-producing thermophilic bacteria have been successfully carried out at the Pariangan hot spring, West Sumatra, Indonesia. Bacterial screening results obtained S2.3 bacterial isolates with the highest activity but the type of bacterial isolates S2.3 is unknown. The purpose of this study was to determine the type of bacterial isolate S2.3 originating from the Pariangan hot spring, West Sumatra, Indonesia using 16S rRNA gene analysis. The 16S rRNA gene is used in bacterial identification because it contains a very evolutionarily conservative sequence. Highly conservative regions can be used as primer attachment sites so that they can be amplified in vitro by PCR. In this way we can study the genetic diversity of an environment in more detail because microbes that cannot be cultured can also be obtained for the 16SrRNA gene [5].

2. MATERIALS AND METHODS

2. 1. Materials

The materials used in this research are Genomic DNA isolation kit, DNA isolation kit, Nutrient Agar, 1 x TE buffer, Sodium Acetate, buffer ekstraksi go Tag, and 70% Ethanol.

2. 2. Methods

2. 2. 1. Genomic DNA Isolation

Isolation of the bacterial genome DNA was carried out by rinsing the bacterial suspension using 500 µl TE buffer 1x and centrifuged at a speed of 13.000 rpm for 5 minutes. The resulting pellet was suspended with 50 µl TE buffer 1x, 300 µl extraction buffer, then mixed using vortex for 5 minutes. 150 µl 3M sodium acetate was added to the suspension and incubated at room temperature for 10 minutes. The suspension was centrifuged for 5 minutes at a speed of 13.000 rpm. The supernatant is transferred into a new micro-tube and added with isopropanol with the same volume (1: 1). The mixture is turned over briefly and centrifuged at a speed of 13.000 rpm for 10 minutes. The resulting pellet was washed with 70% ethanol then centrifuged for 1 minute at a speed of 13.000 rpm. The resulting genome or pellet DNA was dried and aerated again with 50 µl TE buffer 1x.

2. 2. 2. DNA Isolation

Amplification of the 16S rRNA gene was carried out using GoTaq (Promega) with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5'-GGTTACCTTGTTACGACTT-3') [6]; (7]. PCR conditions were set as many as 30 cycles with a predenaturation temperature of 94 °C for 5 minutes, denaturation of 94 °C for 45 seconds, annealing 50 °C for 45 seconds, extension72 °C for 45 seconds, and post extension 72 °C. Data sequencing results were then processed with the crustal X program, Bioedit, and MEGA 6.

2. 2. 3. Analysis of the 16S rRNA gene

The 16S rRNA genes were sequenced in BLASt and compared with16S the rRNA gene is another bacterium that has been deposited in Genbank. Alighment of sequential data is carried out with the Crustal X program. Alignment begins by inputting data into the Crustal X file in the form of Fasta and carrying out alignment. Alignment reults are then edited with the Bioedit program. Phylogenetic trees are created based on DNA sequences using the MEGA6 program. The MEGA6 program is based on several statistical methods, namely the distance method, the parsimony method, and the likelihood method. In the distance method, the evolutionary distance is calculated for all pairs of taxa and phylogenetic trees based on the relationship between

distance values. The maximum parsimony is based on the smallest number of nucleotide substitutions which explains the entire evolutionary process for making phylogenetic trees. Phylogenetic trees are best when the number of substitutions is small. The maximum likelihood i based on calculating the number of possible site variation patterns produced by the substitution process and the observed base frequencies.

3. RESULTS

DNA isolation results of bacterial isolate S2.3 indicate the presence of DNA bands, which means that the genomic DNA was successfully isolated [Figure no 1].

Figure no 1. DNA isolation results of bacterial isolate S2.3

The amplification results show the presence of a DNA band measuring 1500 bp [Figure no 2]. PCR uses 16S rRNA primers with primer pairs of 27F and 1492R for 30 cycles.

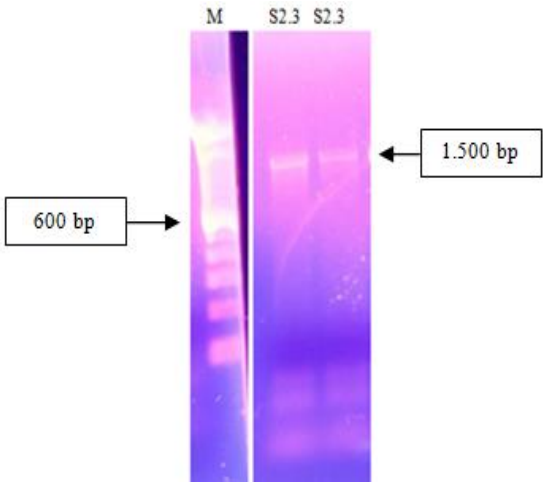

Figure no 2. The amplification results (M= marker 100bp; S2.3 = bacteria isolate

The relationship between 11 bacterial isolates and S2.3 bacterial isolates showed that S2.3 bacterial isolates had a close relationship with the *Bacillus licheniformis* strain ATCC 14580 with genetic distance values (p-distance = 0.002) and a similarity or homology level of 98% [Figure no 3].

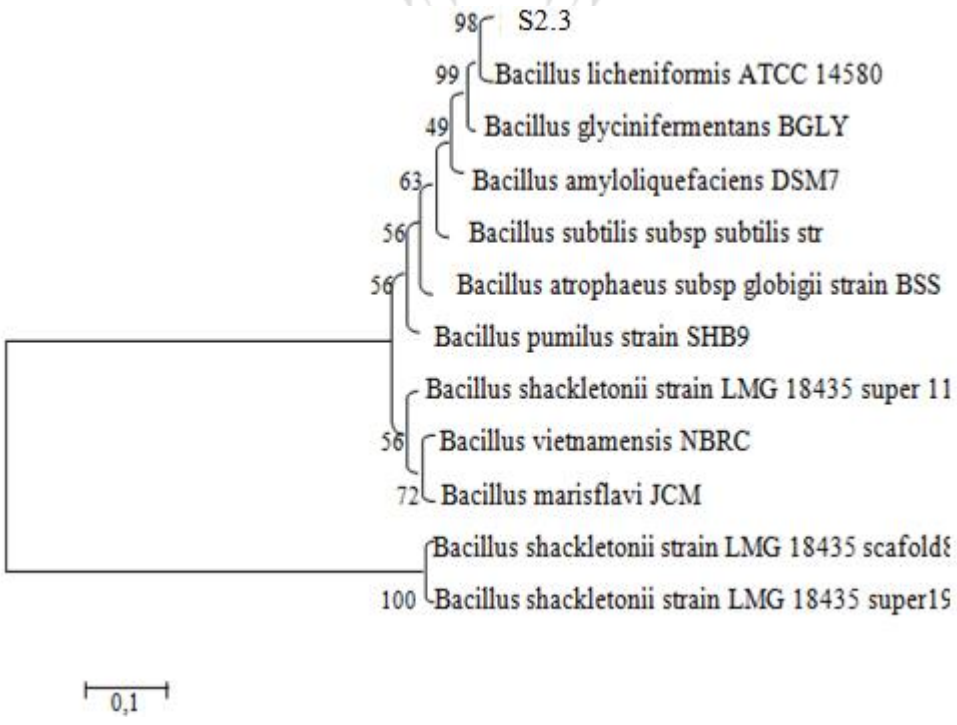

Figure no 3. Phylogenetic trees using the MEGA6 program

4. DISCUSSION

Results of 16s rRNA gene sequencing were compared with DNA sequences from 11 bacteria deposited in Genbank through the BLAST (*Basic Local Alignment Search Tool*) program. BLAST results have a 98% similarity value. The value of genetic distance or nucleotide difference between isolates (p-distance) is 0,000-1,127. DNA is the whole set of chromosomes owned by bacteria and is generally large and less variable in some species when compared to the genome of eukaryotes. Genomic DNA isolation aims to obtain bacterial genomes that will be used at the working stage of gene amplification. The intensity of the DNA band of the 16S rRNA PCR product is visible, this shows that the template DNA concentration and PCR conditions used are suitable for amplification of the 16S rRNA genes. The intensity of the DNA band of the 16S rRNA gene PCR product from bacterial isolates IMB-9 and IMB-10 was not apparent because the DNA concentration of the template used was small [8].

So it can be stated that the bacterial isolate S2.3 is *Bacillus licheniformis* strain ATCC 14580 located in the Pariangan hot spring, West Sumatra, Indonesia. This bacterium can produce amylase which plays a role in industries, especially as an additive detergent. *Bacillus licheniformis* strain ATCC 14580 is a Gram-positive bacterium, has spores, and lives in soil, water, and plants. *Bacillus licheniformis* amylase has been isolated and identified from hot springs Jordan [9). Amylase is generally a metalloenzyme that requires calcium and chloride ions for activity and maintains the stability and integrity of the amylase structure [10]. Amylase will cut the α-1.4 glycosidic bonds in starch molecules (carbohydrates) to form shorter carbohydrate molecules.

5. CONCLUSION

The results of 16S rRNA gene amplification of the S2.3 bacterial isolate showed a DNA band measuring 1500 bp. Based on the results of BLAST, it showed that the isolate bacterium S2.3 had a 90% similarity with the *Bacillus.*

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