ISOLASI DAN IDENTIFIKASI FRAGMEN GEN 16S rRNA

BAKTERI TERMOFILIK ANAEROB SUMBER AIR PANAS GEDONG SONGO KAWAH 1 (GS 1) AMBARAWA

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RINGKASAN

Penelitian mengenai bakteri termofilik anaerob di Jawa Tengah masih terbatas pada isolasi enzim tanpa identifikasi jenis bakteri. Identifikasi bakteri termofilik anaerob penghasil enzim termostabil dilakukan untuk mengetahui jenis bakteri, kekerabatan antarbakteri dan keberadaan bakteri baru. Identifikasi juga bermanfaat untuk mengetahui enzim termostabil yang dihasilkan agar dapat digunakan untuk berbagai keperluan. Bakteri termofilik anaerob Gedong Songo kawah 2 (GS 2) Ambarawa telah diisolasi dan diidentifikasi yang memiliki kemiripan dengan *Pseudomonas synxantha*. Penelitian ini bertujuan untuk memperoleh isolat bakteri termofilik anaerob dari sumber air panas Gedong Songo kawah 1 (GS 1) Ambarawa dan mengidentifikasi jenis bakteri berdasarkan uji mikrobiologi, analisis gen 16S rRNA dan uji secara kualitatif potensi enzim ekstraseluler.

Isolasi bakteri termofilik anaerob dari sumber air panas GS 1 dilakukan dengan variasi media SP (*Sucrose Peptone*), LMM (*Lactose Minimal Media*) dan LMMY (*Lactose Minimal Media Yeast*) dengan variasi temperatur dan waktu pertumbuhan. Identifikasi bakteri termofilik anaerob dilakukan dengan uji fenotipik, genotipik, dan identifikasi enzim ekstraseluler. Uji fenotipik yaitu pengujian secara mikrobiologi, meliputi pewarnaan gram dan morfologi. Uji genotipik yaitu analisis gen 16S rRNA dengan metode PCR dan sekuensing. DNA kromosom bakteri sampel diisolasi dan diamplifikasi secara *in vitro* dengan metode PCR. Penentuan urutan nukleotida fragmen 16S rRNA dilakukan dengan metode sekuensing. Kemudian hasilnya dibandingkan dengan *database GenBank* untuk mengetahui jenis bakteri sampel. Identifikasi enzim ektraseluler menggunakan media SP dengan penambahan gelatin (uji protease), pati (uji amilase), dan laktosa (uji ?-galaktosidase).

Hasil penelitian menunjukkan bahwa bakteri sampel memiliki temperatur optimal pertumbuhan 65°C dan waktu optimal pertumbuhan selama 4 hari. Uji mikrobiologi menunjukkan bahwa bakteri sampel termasuk bakteri gram negatif dan berbentuk batang (*bacillus)*. Bakteri sampel memiliki kemiripan genotipik dengan *Pseudomonas synxantha* sebesar 99 %, serta memiliki persamaan sifat fenotifik kecuali pada bentuk morfologinya. Bakteri sampel memiliki kemiripan fenotifik secara keseluruhan dengan *Pseudomonas aeruginosa* dengan kemiripan genotipik sebesar 91%. Berdasarkan hasil analisis program *Primer Select*-DNASTAR, tidak terjadi penempelan primer PCR BacF1 dan UniB1 pada gen 16S rRNA *Pseudomonas synxantha* maupun *Pseudomonas aeruginosa*. Maka bakteri sampel yang diperoleh berpotensi sebagai bakteri termofilik anaerob jenis baru (novel) dan temasuk dalam satu genus dengan bakteri yang didapat dari GS 2. Enzim ekstraseluler yang dihasilkan yaitu enzim amilase, protease, dan ?-galaktosidase.

SUMMARY

The researches on anaerob thermophilic bacteria in Central Java are only limited to enzyme isolation without species bacteria identification. The identification of anaerob thermophilic bacteria which produce thermostable enzyme is to know species of bacteria, bacteria phylogeny, and novel bacteria. The identification is also aimed to know the termostable enzyme produced that applicable for any applications. Anaerob thermophilic bacteria from Gedong Songo hot spring 2 (GS 2) Ambarawa had been isolated and identified that the bacteria is similar with *Pseudomonas synxantha***.** The purpose of this research is to get the isolate from Gedong Songo hot spring 1 (GS 1) Ambarawa and to identify the species of bacteria based on microbiology test, 16S rRNA gene analysis and qualitative extracelluler enzyme identification.

Isolation of anaerob thermophilic bacteria from Gedong Songo hot spring 1 (GS 1) Ambarawa had been done in SP (Sucrose Peptone), LMM (Lactose Minimal Media) and LMMY (Lactose Minimal Media Yeast) medium with variation of temperature and time of living, followed by phenotypic and genotypic test, and extracelluler enzyme identification. Before genotific test, sampel bacteria's chromosomal DNA was isolated and *in vitro* amplified with PCR method. Nucleotide sequences from 16S rRNA fragment were determined with sequencing method. Then the result were compared with those in database from GenBank to identify bacteria's species sample. Extracelluler enzyme identification used SP medium with adding gelatin (protease test), starch (amylase test) and lactose (?-galactosidase test).

The result of research showed that sample of bacteria had optimum growth temperature of 65°C and optimum growth at 4 days. Microbiology test showed that bacteria sample was gram's negative and had bacillus shape. Bacteria sample had 99% genotype similarity with *Pseudomonas synxantha* and had phenotypic similarity except the morphology shape. Sample bacteria had all phenotypic similarity with *Pseudomonas aeruginosa* and had 91% genotype similarity. Based on analysis of Primer Select-DNASTAR program, there are no anneal side BacF1 and UniB1 PCR primer compatible at 16S rRNA gene of *Pseudomonas synxantha* and *Pseudomonas aeruginosa*. Therefore the sample bacteria had a potency as novel thermophilic anaerobic bacteria and it had same genus with bacteria from GS 2. Extracelluler enzymes produced were amylase, protease, and ?-galactosidase enzymes.

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