IDENTIFIKASI BAKTERI TERMOFILIK ASIDOFIL

DARI ISOLAT KAWAH SIKIDANG DIENG JAWA TENGAH

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RINGKASAN

Bakteri termofilik asidofil merupakan mikroorganisme yang dapat tumbuh pada temperatur tinggi dan pH rendah. Bakteri ini memiliki sebaran yang luas. Akan tetapi bakteri yang sudah teridentifikasi jumlahnya masih terbatas. Pada penelitian ini dilakukan identifikasi terhadap bakteri termofilik asidofil isolat dari Kawah Sikidang, Dieng, Jawa Tengah.

Analisis dilakukan secara mikrobiologi (uji morfologi dan enzim ekstraseluler) dan genetika molekul. Sampel bakteri diisolasi dan dikulturkan dalam medium A dan medium B. Molekul DNA kromosom sampel bakteri diekstraksi dan dimurnikan selanjutnya diamplifikasi dengan metode PCR (*Polymerase Chain Reaction*). Varian amplikon fragmen gen 16S rRNA dipisahkan dengan SSCP (*Single-Strand Conformation Polymorphism*), kemudian ditentukan urutan nukleotidanya dengan sekuensing. Pada tahap akhir, dilakukan komparasi dengan *data base GenBank.*

Sampel bakteri merupakan Gram-negatif berbentuk kokus yang dapat tumbuh optimum pada temperatur 65 °C (AD3) dan 70 °C (BD3). Kedua sampel tersebut dapat menghasilkan enzim protease ekstraseluler. Pada analisis PCR, fragmen gen 16S rRNA dapat teramplifikasi sebesar 353 pasang basa (pb). Pola spesifik SSCP sampel AD3 menunjukkan adanya dua spesies bakteri, sedangkan sampel BD3 mempunyai pola *smear.* Analisis data sekuensing spesies spesifik sampel AD3 menunjukkan adanya homologi sebesar 98 % dengan *Pseudomonas fluorecens* strain Pf1 dan perbedaan urutan sebesar 2 % disebabkan oleh adanya substitusi di lima tempat yaitu pada posisi basa 1071, 1074, 1076, 1357, 1358 dan insersi pada 1125.

SUMMARY

Thermophilic acidofile are microorganisms that growth at elevated temperature and low pH. These bacteria have a large spreading, but the identified bacteria are small. This research was done to identify of thermophilic acidofile bacteria isolat of Sikidang Crater's, Dieng, Central Java.

Molecular genetic analysis and microbiologycal methods (morphology and extracellular enzyme) have used to identify bacteria. Sample bacteria was isolated and cultured on medium A and medium B. Chromosomal DNA was extracted and purified from cultured sample bacteria and 16S rRNA genes fragment was amplified by PCR (Polymerase Chain Reaction) methode. The variant of amplicon 16S rRNA genes fragment was separated by SSCP Single-Strand Conformation Polymorphism) and then determination the nucleotides sequences of 16S rRNA genes fragment using sequencing. Finally, sequencing data was compared with data base from GenBank.

Sampel bacteria are Gram-negative and coccus that growth at optimum temperature 65 °C (AD3) dan 70 °C (BD3). Both sampel AD3 and BD3 can produced protease extracellular enzyme. On PCR analysis, 353 base pairs of 16S rRNA genes fragment can be amplified. Species spesifics SSCP pattern of sample AD3 were determined for two species and SSCP pattern of sample BD3 were smearing. Sequencing data analysis of species spesific sample AD3 showed that obtained 98 % homology with *Pseudomonas fluorecens* stain Pf1 and 2 % different sequence caused by substitution at base position 1017, 1074, 1075, 1357, 1358 and insertion at 1125 position.

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