INTISARI

Bacillus anthracis adalah patogen penyebab antraks, yang menghabiskan sebagian besar siklus hidupnya sebagai spora, yang dapat bertahan lama pada berbagai jenis tanah. Bentuk dorman menjadikan *B. anthracis* memiliki sedikit variasi susunan genetik yang secara mikrobiologi konvensional belum dapat mengungkapkan karakter berbagai isolat. Kasus antraks yang disebabkan *B. anthracis* masih ditemukan di daerah endemis Propinsi Jawa Timur, Jawa Tengah, dan Daerah Istimewa Yogyakarta (DIY). Penelitian ini bertujuan untuk mengidentifikasi karakter fenotipe, genotipe dan kekerabatan, serta virulensi *B. anthracis* isolat asal propinsi Jawa Timur, Jawa Tengah, dan DIY. Sampel yang digunakan pada penelitian ini berjumlah 29 *B. anthracis* isolat lapang, serta isolat kontrol *B. anthracis strain* vaksin (Sterne 34F2) dan *B. cereus* ATCC 11778. Semua isolat diidentifikasi secara fenotipe melalui uji kultur pada media plat agar darah (PAD), media bikarbonat 0,7% dengan CO_2 5%, pewarnaan Gram, pewarnaan kapsul (M'Fadyean), dan pewarnaan spora (modifikasi *Ziehl-Neelsen*). Isolat dikonfirmasi menggunakan *polymerase chain reaction* (PCR) multipleks terhadap marker kromosomal *Ba813*, plasmid gen *lef* (pXO1), dan gen *capC* (pXO2). Selanjutnya genom utuh isolat disekuensing dengan teknik *Next Generation Sequencing* (NGS), dan dibandingkan dengan genom rujukan Ames Ancestor (*GenBank Accession No.* AE017334.2; AE017336.2; AE017335.3). Data penelitian meliputi sifat fenotipe, genotipe, dan virulensi dianalisis secara deskriptif. Hasil penelitian didapatkan sebanyak 27 (93,10%) isolat koloni berwarna putih-keabuan, permukaan kasar tepi bergelombang, tidak menghemolisis eritrosit, koloni mukoid pada media bikarbonat, non-motil, sensitif terhadap antibiotik penisilin dan tetrasiklin. Sebanyak 2 (6,89%) isolat tidak berkapsul, berwarna kekuningan dengan pusat koloni menonjol *(umbonate)*, permukaan koloni halus sedikit mukoid pada media PAD, non-mukoid pada media bikarbonat, dan 1 dari 2 isolat tersebut menunjukkan sifat resisten terhadap penisilin. Isolat dengan koloni mukoid mengamplifikasi gen *Ba813*, *capC*, dan *lef* pada uji PCR multipleks, sedangkan isolat koloni non-mukoid hanya mengamplifikasi gen *Ba813* dan *lef*. Berdasarkan hasil pemeriksaan tersebut, 29 isolat diidentifikasi sebagai *B. anthracis*. Salah satu isolat *B. anthracis* virulen memiliki 1 genom kromosom berukuran 5.226.658 bp, dan 2 plasmid yaitu pXO1 berukuran 181.677 bp dan pXO2 berukuran 94.830 bp dan memiliki kemiripan >90% dengan *strain* rujukan. Analisis SNP terhadap 11 isolat diperoleh 2 tipe SNP yang memisahkan isolat asal Jawa Tengah dengan isolat Jawa Timur dan DIY, namun semua isolat memiliki kekerabatan yang dekat dari jarak SNP yang pendek (1-8 SNP). Disimpulkan bahwa isolat yang diuji memiliki kekerabatan yang dekat dimana 27 (93,10%) merupakan *B. anthracis strain* virulen, dengan fenotipe mukoid dan berkapsul, sedangkan 2 (6,89%) *strain* avirulen dengan fenotipe non mukoid dan tidak berkapsul.

Kata Kunci: *Bacillus anthracis*, fenotipe, genotipe, virulensi.

ABSTRACT

Bacillus anthracis is a pathogen causing anthrax in which it spends most of its life cycle as the spore that is able to survive for a long time in various soil types. The form of dormant has made *B. anthracis* has a slight variation in genetic composition which in conventional microbiology has not been able to reveal the characters of various isolates. The cases of anthrax caused by *B. anthracis* are still found in endemic areas of East Java, Central Java, and the Special Region of Yogyakarta (DIY). This study aims to identify the phenotypes, genotypes, and phylogenetic relationships, as well as the virulence of *B. anthracis* isolates from the provinces of East Java, Central Java, and DIY. The samples used in this study were 29 field isolates of *B. anthracis*, as well as control isolates of *B. anthracis* vaccine strain (Sterne 34F2) and *B. cereus* ATCC 11778. All isolates were identified phenotypically by culture test on the media of sheep blood agar (SBA), 0.7% bicarbonate medium with a 5% CO2, Gram staining, capsule staining (M'Fadyean), and spore staining (*Ziehl-Neelsen* modification). The isolates were then identified molecularly using *multiplex polymerase chain reaction* (PCR) against chromosomal marker *Ba813, lef* gene plasmid (pXO1), and *capC* gene (pXO2). Furthermore, the whole genome of the isolate was sequenced using the *Next Generation Sequencing* (NGS) technique and compared with the reference genome of Ames Ancestor (*GenBank Accession* No. AE017334.2; AE017336.2; AE017335.3). Research data including phenotype, genotype, and virulence were analyzed descriptively. The results showed 27 (93.10%) isolates of gray-white colonies, rough surface with wavy edges, not hemolyzing erythrocytes, mucoid colonies on bicarbonate media, non-motile, and sensitive to penicillin and tetracycline antibiotics. A total of 2 (6.89%) isolates were not encapsulated, yellowish with a prominent colony center (*umbonate*), the surface of the colonies was smooth, slightly mucoid on PAD media, non-mucoid on bicarbonate media, and 1 of the 2 isolates showed penicillin resistance. Isolates with mucoid colonies amplified *Ba813, capC,* and *lef* gene in the multiplex PCR assay, while isolates with non-mucoid colonies only amplified *Ba813* and *lef* gene. Based on the results of these examinations, 29 isolates were identified as *B. anthracis*. One of the virulent *B. anthracis* isolates had 1 chromosomal genome in the size of 5,226,658 bp, and 2 plasmids, namely pXO1 with the size of 181,677 bp and pXO2 with the size of 94,830 bp and having >90% similarity with the reference strains. The SNP analysis of 11 isolates obtained 2 types of SNP sparating the isolates from Central Java from East Java and DIY isolates. But all isolates were closely related from short SNP distances (1-8 SNP). In conclusion, the isolates tested were closely related where 27 (93,10%) were virulent strain of *B. anthracis*, with a mucoid colony phenotype and had capsules, while 2 (6,89%) were avirulent strains with non-mucoid and non-capsulated colony phenotypes.

Key words: *Bacillus anthracis*, phenotype, genotype, virulence.