

# Identifikasi dan Analisis Filogenetik *Aspergillus* section *Nigri* sensu Gams et al., 1985 pada Rhizosfer Marga Piper di Kebun Raya Eka Karya, Bedugul Bali = Identification and Phylogenetic Analysis of *Aspergillus* section *Nigri* sensu Gams et al. 1985 Inhabiting Piper Rhizosphere from Eka Karya Botanical Garden, Bedugul Bali

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## Abstrak

*Aspergillus* section *Nigri* adalah salah satu kelompok kapang yang memiliki peran penting dalam bidang mikologi pangan, kedokteran, dan bioteknologi. Kapang tersebut merupakan kandidat yang sering digunakan untuk rekayasa genetika dan pemerintah Amerika Serikat melalui Food and Drug Administration (FDA) memberikan status GRAS (Generally Regarded As Safe) dalam penggunaannya di bidang industri dan bioteknologi. Secara sistematika dan taksonomi, kapang *Aspergillus* section *Nigri* memiliki sejumlah permasalahan karena kapang tersebut sukar untuk diidentifikasi dan diklasifikasi. Dalam penelitian ini dilakukan identifikasi dan analisis filogenetik terhadap 20 strain *Aspergillus* section *Nigri* terseleksi asal Kebun Raya Eka Karya, Bedugul Bali. Identifikasi kapang terseleksi dilakukan melalui pendekatan morfologi dan molekuler. Karakterisasi morfologi dilakukan dengan mengamati karakter fenotip di media CzA, MEA, CYA, MEA37, dan CY20S. Adapun analisis molekuler dilakukan melalui analisis sekuensing gen pada lokus ITS rDNA, gen  $\beta$ -tubulin dan calmodulin. Analisis filogenetik dilakukan menggunakan analisis statistik neighbor-joining (NJ). Hasil analisis morfologi dalam penelitian ini belum dapat digunakan untuk mengidentifikasi dan membedakan ke-20 strain pada tingkat takson spesies. Hasil analisis molekuler menunjukkan 7 strain memiliki kedekatan secara genotip dengan *A. aculeatus* pada kisaran homologi 97-99%, 4 strain memiliki kedekatan dengan *A. niger* pada kisaran homologi 99-100%, dan 9 strain memiliki kedekatan genotip dengan *A. tubingensis* pada kisaran homologi 97-100%. Hasil analisis molekuler juga menunjukkan 10 strain yaitu P03, P08, P09, P10, P12, P15, P16, P18, P19, dan P20 memiliki homologi yang rendah pada lokus gen  $\beta$ -tubulin dan calmodulin sehingga secara genotip strain tersebut kemungkinan merupakan kandidat spesies yang berbeda. Hasil tersebut diperkuat oleh hasil analisis filogenetik NJ pada ketiga lokus. Berdasarkan hasil analisis filogenetik multilokus strain P01, P02, P11, dan P17 adalah takson *A. tubingensis*, strain P04, P05, P06, dan P07 adalah takson *A. niger*, dan strain P13 dan P14 adalah takson *A. aculeatus*. Hasil analisis filogenetik juga menunjukkan adanya spesies tersembunyi (cryptic species) dari beberapa strain *Aspergillus* hitam yang disolasi dari rhizosfer Piper asal Kebun Raya Eka Karya, Bedugul Bali, yaitu strain P03, P08, P09, P10, P12, P15, P16, P18, P19, dan P20.

.....The black aspergilli (*Aspergillus* section *Nigri*) are an important group of species in food mycology, medical mycology, and biotechnology. They are also candidates for genetic manipulation in the biotechnology industries since *A. niger* used under certain industrial condition has been granted the GRAS (Generally Regarded As Safe) status by the Food and Drug Administration of the US government. Black aspergilli are one of the more difficult groups regarding classification and identification. In spite of the taxonomy of the *Aspergillus* species of the *Nigri* section being regarded as troublesome. This work aimed to identify and analyse the phylogeny of 20 selected strains of black aspergilli from Eka Karya Botanical Garden, Bedugul Bali. Morphological character were observed from culture were grown on CzA, MEA,

CYA, MEA37, and CY20S. Meanwhile, molecular analysis have been conducted based on the ITS rDNA,  $\beta$ -tubulin, and calmodulin genes. Morphological data result are useful for preliminary identification but it did not having been totally effective in describing and elucidating 20 selected strains into species level. Further molecular analysis showed that from 20 selected strains, seven strains have 97-99% similarity with *A. aculeatus*, four strains have 99-100% similarity with *A. niger*, and nine strains have 97-100% similarity with *A. tubingensis*. Based on molecular analysis particularly  $\beta$ -tubulin and calmodulin genes, 10 strains (P03, P06, P08, P09, P10, P12, P15, P16, P18, P19, and P20) can be presumed as new species because of the low homology value to their closest related species. Based on the phylogenetic analysis strains of P01, P02, P11, and P17 were identified as *A. tubingensis*; strain P04, P05, P06, and P07 were identified as *A. niger*, and strain P13 and P14 were identified as *A. aculeatus*. Ten strains, namely, P03, P08, P09, P10, P12, P15, P16, P18, P19, and P20, form distinct lineage separated from other recognized *Aspergillus* in this section. Cryptic species probably exist among the *Aspergillus* section *Nigri* strains inhabiting *Piper* rhizosphere from Eka Karya Botanical Garden, Bedugul Bali.