

**BACTERIAL COMMUNITIES ASSOCIATED
WITH TOXIC AND NON-TOXIC MARINE
DINOFLAGELLATES**

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BACTERIAL COMMUNITIES ASSOCIATED WITH TOXIC AND NON-TOXIC MARINE DINOFLAGELLATES

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Association between bacteria and marine dinoflagellates is one of the important factors in harmful algae bloom formation and toxin production by dinoflagellates. The diversity of the bacteria associated with clonal cultures of both toxic and non toxic dinoflagellates, *Alexandrium tamiyavanichii* and *Alexandrium leei* were assessed by using culture dependent and culture independent approaches. In culture-dependent approach, bacteria were isolated on appropriate culture media and later identified based on 16S rDNA sequences. In culture-independent approach metagenome was extracted directly from the cultures of dinoflagellates. The extracted nucleic acid were then used for 16S rDNA amplification. The amplicon were cloned into plasmid vector. Plasmids from constructed libraries were purified and sequenced. Nucleotide sequences obtained were then compared with sequences from the GenBank Database. 16S metagenomics sequencing was also carried out. Sequences were then assembled and classified to elucidate the associated bacteria communities. A total of 17 bacteria were isolated from culture of *Alexandrium tamiyavanichii* and 21 were from *Alexandrium leei*. 16S rDNA sequences analysis revealed that bacteria associated with two species of dinoflagellates were a diverse group of *Alpha-Proteobacteria* (α) (40%) followed by *Gamma-Proteobacteria* (γ)

and *Cytophaga-Flavobacter-Bacteroides* (CFB) (21%), and *Firmicutes* (14.7%). In 16S PCR cloning and sequencing analysis, a total of 50 phlotypes were obtained from the directly amplified DNA of both cultures, of which 22 phlotypes were obtained from *Alexandrium tamiyavanichii* and 28 from *Alexandrium leei*. Sequence analysis of the clones also revealed that associated bacteria belonged to α -*Proteobacteria* (48.4%), CFB (21.2%), unknown bacteria group (18.9%) and γ -*Proteobacteria* (10.5%). In addition, one phlotype belonged to *Planctomycetes* was discovered from culture of *Alexandrium leei*. Bacteria community structure by 16S metagenomic analysis also revealed that both samples of dinoflagellates were mainly dominated by *Proteobacteria* phyla (69.3%), followed by *Bacteroidetes*, (19.85%) Phyla of *Fusobacteria* (2.62%), *Actinobacteria* (4.65%), and *Firmicutes* (2.62%) were also found to be minority communities. The results suggest that a number of different bacterial species are associated with dinoflagellates, some of which are common to each of the dinoflagellate cultures examined, whereas others appear to be unique to a particular dinoflagellate. Present study showed that culture-independent method is necessary to capture the full diversity of these associated bacteria since a large percentage of uncultured bacteria sequences were obtained.

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**KOMUNITI BAKTERIA YANG BERSEKUTU DENGAN DINOFLAGELAT
MARIN BERACUN DAN TIDAK BERACUN**

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Hubungan antara bakteria dan dinoflagellat marin adalah salah satu faktor penting dalam pembentukan ledakan populasi alga dan penghasilan toksin oleh dinoflagellat. Kepelbagaian bakteria yang bersekutu dengan kultur dinoflagellat beracun, *Alexandrium tamiyavanichii* dan kultur tidak beracun, *Alexandrium leei* ditentukan dengan menggunakan kaedah melibatkan pengkulturan dan kaedah tanpa pengkulturan. Dalam pendekatan melibatkan pengkulturan, bakteria telah dipencilkan dan dikenalpasti berdasarkan jujukan nukleotida 16S rDNA. Dalam pendekatan tanpa pengkulturan pula, metagenom telah diekstrakkan secara langsung daripada kultur-kultur dinoflagellat. Asid nukleik yang terekstrak kemudian digunakan untuk amplifikasi 16S rDNA. Amplikon terjana telah diklonkan menggunakan vektor plasmid. Plasmid daripada perpustakaan terbina telah dituliskan dan diujuk. Jujukan nukleotida yang diperolehi kemudian dibandingkan dengan jujukan sedia ada daripada pangkalan data GenBank. Penjujukan metagenomik 16S juga telah dilakukan. Jujukan-jujukan terjana kemudian dicantum dan dikelaskan untuk menentukan komuniti bakteria yang bersekutu. Sebanyak 17 bakteria telah dipencilkan daripada *Alexandrium tamiyavanichii* dan 21 bakteria

daripada *Alexandrium leei*. Analisis 16S rDNA telah mendedahkan bahawa bakteria yang bersekutu dengan kedua dua spesies dinoflagellat adalah terdiri daripada filum α -Proteobakteria (40%) diikuti oleh γ -Proteobakteria dan Cytophaga-Flavobacter-Bacteroides (CFB) (21%) dan filum Firmicutes (18%). Dalam PCR klon 16S dan analisis penjujukan, sejumlah 50 filotip diperolehi, di mana 22 filotip diperolehi daripada *Alexandrium tamiyavanichii* dan 28 daripada *Alexandrium leei*. Jujukan analisis klon telah mendedahkan kumpulan utama bakteria yang berasosiasi dengan kedua dua spesies dinoflagellat iaitu, kumpulan α -Proteobakteria(48.4%), CFB (21.2%), kumpulan bakteria yang tidak diketahui (18.9%) dan γ -Proteobakteria (10.5%). Sebagai tambahan, satu filotip dari kumpulan Planctomycetes telah dijumpai daripada *Alexandrium leei*. Analisis struktur komuniti bakteria bersekutu menggunakan analisis metagenomik 16S telah mendedahkan bahawa kedua-dua sampel dinoflagellates kebanyakannya dikuasai oleh filum Proteobakteria (69.3%), diikuti oleh Bacteroidetes (19.85%), filum Fusobacteria (2.62%), Actinobacteria (4.65%) dan Firmicutes (2.62%) juga dijumpai membentuk komuniti minoriti. Keputusan menunjukkan bahawa beberapa spesies bakteria yang berbeza yang berkaitan dengan dinoflagellat, ada juga yang terdapat pada setiap kultur dinoflagellat, manakala yang lain hanya terdapat dan unik kepada dinoflagellat tertentu. Kajian ini menunjukkan bahawa kaedah tanpa pengkulturan adalah perlu untuk memperoleh keputusan kepelbagaian persekitaran bakteria berasosiasi dengan dinoflagellat dimana kebanyakan daripada bakteria diperolehi melalui kaedah tanpa pengkulturan.