Production and Characterization of Biosurfactants from Cyanobacteria
シアノバクテリア由来の界面活性物質の探索

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[Introduction] Biosurfactants are promising amphipathic compounds derived from microorganisms. They have advantages compared with synthetic surfactant because of their higher biodegradability, lower toxicity and effectiveness at extreme environment. Because of the unique structure and diverse properties, biosurfactant exhibit a large number of bioactivities including anticancer, antimicrobial, anti-adhesive and anti-biofilm activity against human pathogens. The high structural diversity and bioactivities of cyanobacteria presents them as potential candidates for future uses in new generations of therapeutic agents. This research aims to identify and characterize the biosurfactant production activity by cyanobacteria.

[Methods] In the present study both marine and freshwater cyanobacteria were screened for their biosurfactant production activity. Total 64 freshwater cyanobacterial extracts were tested by oil spreading assay and 5 freshwater cyanobacteria strain were tested for their biosurfactant activity during culture period by measuring the surface tension. Marine samples collected from Sabah, Malaysia were identified using 16S rRNA gene sequencing analysis. The chemical profiling of lipophilic fractions were done by liquid chromatography mass spectrometry (ESI-LC-MS) analysis. The crude ethyl acetate fraction was separated by silica gel column chromatography and the target compounds were purified using reversed phase high performance liquid chromatography (HPLC). The structure elucidation of secondary metabolites from marine cyanobacteria was determined by NMR and mass spectroscopy analysis and the bioactivity test was conducted by oil spreading assay for biosurfactant activity and *in vitro* MTT assay for cytotoxicity.

[Results and Discussion] The results of hydrophilic fractions showed that, of total 64 freshwater cyanobacterial extracts, 62% (40 cyanobacteria) exhibited biosurfactant activity. The cultured freshwater cyanobacteria strain *Dolichospermum affine* (NIES 40) and *Dolichospermum* sp. (NIES 1697) showed reduction in surface tension of culture medium around 53 mN/m. The diameter of the oil clearing zone of the crude biosurfactant produced by *D. affine* (NIES 40) was 88 mm; its critical micelle concentration (CMC) was approximately 1140 mg/L. Six samples of marine cyanobacteria were identified by 16S rRNA gene sequencing. The chemical profile of marine cyanobacteria showed richness of secondary metabolites including known cytotoxic compounds apratoxins, wewakazoles, lyngbyabellins, columbamide D and unknown halogenated secondary metabolites. Most of the samples showed potent biosurfactant activity. The known compound apratoxin A, columbamide D and unknown columbamide D type of compound with mass m/z 494.2786 isolated from *Moorea bouillonii* showed potent biosurfactant activity. The diameter of clear zones for apratoxin A, columbamide D and unknown columbamide D type of compound is approximately 100 mm, 110.02 mm and 90.02 mm respectively at concentration 1 mg/mL.