MICROBIAL DIVERSITY IN DISTURBED AND UNDISTURBED PEAT SWAMP FOREST AND ISOLATION OF CYANOBACTERIA

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SUMMARY: Microbial diversity from disturbed and undisturbed peat swamp forest obtained from next generation sequencing. Through this analysis, genera cyanobacteria is being compared with isolated cyanobacteria from both environmments which is extremely acidic. For the future study, this genera has ability as biofertilizer in acidic soil for plantations.

Keywords- Biofertilizer, Cyanobacteria, Microbial Diversity, Next Generation Sequencing, Peat Swamp Forest.

INTRODUCTION

Peatland is an ecosystem where organic matter is degraded from decayed plant which accumulates under wet condition. This ecosystem covers 4 million km^2 globally which represents for 180 countries. However, peatland in Southeast Asia covers 6% of global peatland area which is about 24.8 million hectares [1, 2]. About 68 billion tonnes (14%) of carbon is stored in the peatland of Southeast A sia [2]. Tropical peatland in Malaysia is known as peat swamp forest (PSF) in which it has unique ecosystem where flora and fauna survived in acidic water and waterlogged condition and it plays vital role as carbon storage [2]. In the tropics zone, PSF which contains greatest peat depth and located at low altitudes in the river valley basins, watershreds and subcoastal area of Souteast Asia suggested that this ecosystem support lower species diversity compared to dryland rainforest [3]. Peat soil from tropical area is different between temperate and boreal region where it is originated from woody plant debris under heavy rainfall and high temperature [3,4].

Study on microbial diversity in undisturbed and disturbed PSF due to fire which is able to survive in acidic condition is quite restricted where they could alter some microbial communities in the peatland [5]. Next generation sequencing (NGS) tools used to investigate complex genetic information from microbial communities and identify microbial diversity from different environments [6]. In this study, other than novelty findings obtained from microbial diversity in both extreme environments, genera of cyanobacteria is chosen as focusing genera as they able to survive in extreme conditions which are highly acidic and high light intensity in Malaysia. The new findings of cyanobacteria resist in extreme condition has high possibilities to fix nitrogen and carbon dioxide. Discovery of cyanobacteria in acidic soil could be one of the candidates of biofertilizer in acidic soil which can be implent by oil palm plantations for further research.

2. MATERIALS AND METHODS

2.1 Isolation of Cyanobacteria: 1g of soil samples obtained from undisturbed Raja Musa Peat Swamp Forest Reserved (RMPSFR) and disturbed PSF of Malaysia Airport Agriculture-Horticulture (MAAH), Sepang, Selangor were diluted into 10 ml of ultrapurified water. Then, the samples were inoculated on pH 7 M11 and BG11 media with cycloheximide by using laboratory conditions with the temperature of 28ºC and the light intensities of 10 – 17 umol m^{-2} s^{-1} (morning), 238 – 427 umol m^{-2} s^{-1} (sunny) and 105 – 106 umol m^{-2} s^{-1} (evening). The cyanobacteria was purified for several times.

2.2 Screening at High Light Intensity: The cyanobacteria obtained was screened using the natural condition on pH 7 M11 and BG11 media with the temperature of 33ºC and the light intensities of 39 – 62 umol m^{-2} s^{-1} (morning) , 1472 – 1581 umol m^{-2} s^{-1} (sunny) and 105-106 umol m^{-2} s^{-1} (evening).

2.3 Screening at Low pH: The cyanobacteria which can resist at high light intensity was screened at pH 7, pH 4 and pH 3 on M11 and BG11 media supplemented with cycloheximide. The cyanobacteria which were added into pH 3 and pH 4 BG11 and M11 media [7] was buffered with acetate buffer [8]. They were grown using the natural condition with the temperature of 33ºC and the light intensity of 39 – 62 umol m^{-2} s^{-1} (morning) , 1472 – 1581 umol m^{-2} s^{-1} (sunny) and 105-106 umol m^{-2} s^{-1} (evening).

2.4 Microbial Diversity Analysis: DNA extracted using PowerSoil DNA Isolation Kit according to the manual of MO BIO. DNA was sent to company for NGS [6].

3. RESULTS AND DISCUSSIONS

3.1 Isolation of Cyanobacteria

Table 1 shows cyanobacterial cultures isolated on M11 and BG11 medium obtained from disturbed PSF of Malaysia Airport Agriculture-Horticulture (MAAH), Sepang, Selangor. This shows that cyanobacteria able to survive in

tropical PSF which is acidic condition. This is an agreement with previous study where cyanobacteria still able to survive in acidic lakes (pH 4.1 – 5) [9] in which it usually grew and dominated neutral to alkali conditions [10]. Based on table 1, different species could be found on M11 and BG11 media where *Aphanothece sp.* was found as predominant species on both media with total number 22 isolates from M11 media and 20 isolates from BG11 media. This finding was different from previous study where *Anabaena* was found as the predominant genera from acidic soil in Ambasamudram followed by *Westiellopsis*, *Nostoc*, *Oscillatoria* and other algae species [11].

Table 1 shows cyanobacteria isolates found from disturbed PSF of Malaysia Airport Agriculture-Horticulture (MAAH), Sepang, Selangor.

Type of Media	Cyanobacterial isolates	No. of isolates
M11	*Aphanothece sp.*	22
	Nostoc sp.	2
	Aphanocapsa sp.	7
BG11	*Aphanothece sp.*	20
	Nostoc sp.	1
	Synechococcus sp.	6
	Microsystis sp.	4

3.2 Screening at High Light Intensity

Based on cyanobacterial isolated found on M11 and BG11 media, only two cyanobacterial isolated from M11 media able to resist at high light intensity (figure 1) whereas no cyanobacterial isolates able to grow on BG11 media at high light intensity. This may due to the damage of photosynthetic and cellular components which caused by over-excitation of photosynthetic pigments during the exposure of sulight during sunny day and it occured when the light included with UV wavelength. However, many cyanobacteria able to survive at high solar and UV radiation [12] which support that these two cyanobacterial isoates able to survive at high light intensity.

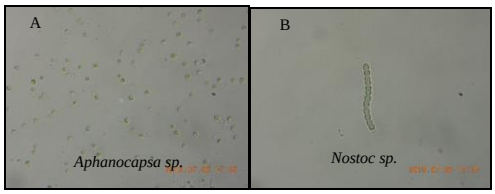

Fig. 1 A and B shows cyanobacteria strains that resist at high light intensity on pH 7 M11 media

3.3 Screening at Low pH

Two cyanobacterial isolates that were able to resist at high light intensity were screened on pH 3 and pH 4 M11 media. However, there was no growth on pH 3 and pH 4 M11 media for both isolates. This shows that both cyanobacterial strains were not able to resist at low pH. Past study also stated that cyanobacteria did not grow below pH 6 [10].

3.4 Microbial Diversity Analysis: DNA was sent to company for NGS. The NGS analysis is still ongoing.

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