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Biodiversity of Indigenous Cyanobacteria In Water Fern, *Azolla* sp., From Malaysian Isolates

Nurul Aina Zulaikha Arbain[a], Rohana Mat Nor[a*]

Structured Abstract

Background: Biodiversity encompasses the variety of life forms across from various ecosystems and it is imperative for ecosystem functioning, economy, including human well-being. *Azolla* is the only known plant with a permanent symbiotic relationship with the nitrogen-fixing cyanobacteria. The plant ability to fix nitrogen has rendered it highly valuable in various economic sectors, including agriculture, pharmaceuticals, and biotechnology. Despite the natural occurrence of *Azolla* in Malaysian water and its widespread agricultural use, there is currently insufficient biodiversity data available for the *Azolla* plant and its cyanobiont. The purpose of this study is to determine the morphological appearance including identifying the species of isolated indigenous cyanobacteria within *Azolla* samples from different locations in Malaysia. The correlation between the number of heterocysts to the health status of *Azolla* was also analyzed in this study.

Methods: Plant tissue squash technique of *Azolla* was adopted to observe cell arrangement patterns and heterocysts. Following that, colony PCR was employed to identify the isolated indigenous cyanobacteria species. *Azolla* samples were respectively grown in vermicompost and NPK chemical fertilizers at several concentrations. Growth performance of *Azolla* was evaluated by calculating its relative growth rate and heterocyst frequency.

Results: All cyanobionts exhibited morphological similarity in terms of cell arrangement patterns and presence of heterocysts. Optimal growth of *Azolla* was observed at specific nutrient concentrations, with vermicompost promoting growth up to 750 ppm, and chemical fertilizer at 250 ppm. Excessive nutrient levels lead to lower growth rates. Both treatments exhibited the highest heterocyst frequency at 250 ppm, triggering heterocyst development for nitrogen fixation. The lowest heterocyst frequency was recorded at 1000 ppm due to the abundance of assimilable nitrogen, inhibiting heterocyst differentiation.

Conclusion: Despite the morphological similarity between the cyanobacteria, attempting to use morphological traits alone is ineffective in achieving a definitive understanding of species identification. Therefore, molecular identification targeting the 16s rRNA region was employed but it encountered significant challenges, particularly during colony PCR. There is also no significant correlation between *Azolla* growth and heterocyst development. Overall, the present findings deepened the understanding on *Azolla*-cyanobacteria association, which might be beneficial for potential biodiversity conservation, climate mitigation and sustainable ecosystem management.

Keywords: Biodiversity, *Azolla*, Indigenous Cyanobacteria, Heterocysts, Malaysia

*Correspondence: rohana_micro@uitm.edu.my

[a] School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia