

Analysis of nitrogen-fixing rhizobacteria associated with *Melastoma malabathricum* L.

Biological nitrogen fixation (BNF) is the process of reducing of atmospheric N₂ gas to biologically available ammonium by nitrogen-fixing bacteria. This process is considered to be the major nitrogen input in nutrient-poor environments. *Melastoma* (*Melastoma malabathricum* L.) is known to grow well in nutrient-poor conditions, however, there is no clear evidence that any BNF is contributing for the growth of this plant. When *Melastoma* was cultured hydroponically without any nitrogen source in an open condition, continuous growth was observed. Furthermore, there was a significant increase in total nitrogen content of the plant. Thus, it is suggested that *Melastoma* has the ability to utilize BNF from atmospheric nitrogen as a nitrogen source.

The objective of this study is to investigate the relationship between *Melastoma* and its associated nitrogen-fixing bacteria.

1. Phylogenetic diversity of nitrogen-fixing rhizobacteria associated with *Melastoma malabathricum* L.

1-1. Comparison of the genetic structure of the diazotrophic community between hydroponic culture medium and in soil.

To reveal whether the hydroponic culture medium where *Melastoma* was cultivated is unusual environment or not, diazotrophic community structure between in hydroponic culture medium and in rhizosphere soil of *Melastoma* will be compared.

Direct DNA extraction_nifH targeted PCR amplification_Construction of nifH clone library

1-2. Comparison of the genetic structure of the diazotrophic community between rhizosphere of *Melastoma* and rice (or other plant)

Check the difference in the structure on *nifH* gene pool between *Melastoma* and rice (or other plant).

Direct DNA extraction_nifH targeted PCR amplification_Construction of nifH clone library.

1-3. Quantification of *nifH* gene in the rhizosphere soil and bulk soil.

Quantification of *nifH* gene by real-time PCR or MPN-PCR (under consideration). Targeted for DNA.

2. Effect of root exudates on nitrogen-fixing activity of rhizobacteria associated with *Melastoma malabathricum* L.

It is assumed that nitrogen-fixing bacteria isolated from *Melastoma* culturing hydroculture media utilize the exudates from *Melastoma* root as carbon source. What components from root exudates do effect on growth of diazotroph associated with *Melastoma* root will be investigated.

2-1. Effect of root exudates to nitrogen-fixing activity.

Characterization and identification of *Melastoma* root exudates under +N and -N condition (Ms.Anda). Addition of root exudate(s) (or crude mixture of exudates) to isolated bacteria particularly found in hydroponic culture solution. Evaluation of nitrogen-fixing activity by ARA.

2-2. Analysis of community structure of diazotroph.

Melastoma were grown hydroponically in nutrient solution with different concentration of nitrogen source (+N and -N). Diazotrophic community structures are compared with different concentration of nitrogen source.

Direct DNA extraction_nifH targeted PCR amplification_Construction of nifH clone library

3. Plant growth-promoting effects of nitrogen-fixing bacteria isolated from rhizosphere of *Melastoma malabathricum* L.

3-1. Inoculation test.

Inoculation of the diazotroph isolates to *Melastoma* grown aseptically in nitrogen-limited condition. Comparing plant growth promoting effect of isolates between gnotbiotic condition and asptical condition by measuring the dry weight and total nitrogen of *Melastoma*.

3-2. Detection of endophyte.

Attempt to detect nitrogen-fixing endophytes from *Melastoma* grown hydroponically and from wild *Melastoma*.