

## MALDI-TOF/MS BASED SCREENING OF PLANT-GROWTH PROMOTING *METHYLOBACTERIUM* SPECIES COLLECTED FROM VARIOUS PLANTS

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### **Abstract**

Plants emit many kinds of volatile organic compounds, including methanol as a main compound. The global methanol emission from plants is estimated at 100 mega tones per year. Recent metagenomic analysis revealed the predominance of *Methylobacterium* species in phyllosphere (plant surface). These species are characterized by their ability to grow on methanol as a sole carbon and energy source. Thus it is considered that such ability is advantageous for these species to grow in relatively nutrient-poor environment as plant surfaces. Also these species are known to have an ability to promote plant growth. They are reported to produce phytohormones (auxin, cytokinin), siderophores, and vitamin B12, and to have ACC deaminase that can inhibit ethylene biosynthesis. Also they have abilities of calcium phosphate solubilization and nitrogen fixation. These abilities are believed to be involved in plant growth promotion. The genus *Methylobacterium* contains 35 known species, and there is an increasing number of new isolates. But the interaction specificities between them and plants, and specificity of plant-growth promotion ability is not clear yet 200 strains of *Methylobacterium* from various plants were collected and subjected to MALDI-TOF/MS analysis for protein profiling of the isolates. The technique allowed identification and clustering of the isolates in a day. The selected unique strains were further subjected to 16S rRNA gene analysis, and their phylogenetic position was revealed. Using the same unique strains, efforts are in placeto find the best combination of strains and plant species. In this presentation I would like to introduce the result of the screening and the effect of inoculation to barley are discussed. As a conclusion, MALDI-TOF/MS-based screening allows maximization of isolates library, fast identification, and selection of unique strains. The technique can be used to find the best strain for specific plants.

**Key words:** Methanol, *Methylobacterium* sp. MALDI-TOF/MS

## 1.0 Introduction

The activities of the microorganisms inhabiting plant surface have great effect on plant growth. Some of them are known to evoke diseases in plants, and others help plant growth through nitrogen fixation, siderophore production, phosphate solubilization, and plant hormone biosynthesis. Utilization of such beneficial microorganisms is expected to make a low-cost and safe biofertilizer. But the mode of interaction between microorganisms and plants has to be understood first.

On the other hand, it is known that the global methanol emission from plants is about 100 mega tones per year. Recent metagenomic analysis revealed the predominance of *Methylobacterium* species in phyllosphere (plant surface), which are capable of growing on methanol as a sole carbon and energy source. Also these species are known to have an ability to promote plant growth. In this study, *Methylobacterium* strains collected from many plant samples were evaluated with high-throughput mass spectrometry-based analysis. Then their ability to promote barley growth was investigated. The result of pot cultivation experiment are here reported.

## 2.0 Materials and Methods

100 samples of plants growing at the Institute of Plant Science and Resources, Okayama University were collected. Those species included rice, wheat, tree, grasses, and mosses. Water suspensions of the samples were spread onto "methanol medium" solidified with agar. The media contained methanol as a sole carbon and energy source. In our screening, pinkish colonies were selectively isolated, and further purified by streaking. A total of 400 bacterial strains were isolated. Methanol assimilation of these isolates was further verified by cultivating them in liquid methanol medium, resulting in final selection of 200 strains. The isolates were then subjected to MALDI-TOF/MS analysis (2).

Bacterial colonies formed on the solid media were picked up with toothpicks, and the bacterial cells were directly smeared onto MALDI target plate. A matrix solution (10 mg/ml Sinappinic acid in 50% acetonitrile plus 2.5% trifluoroacetate) was dropped onto the samples. For mass standards, *Escherichia coli* cells were used because the masses of its ribosomal proteins are known. The spectra of isolates were aligned and analyzed to construct a phylogenetic tree using UPGMA method. The analysis included all the type strains in the genus. The isolates found to be unique in the tree were further selected and subjected to 16S rRNA gene analysis. The 16S rRNA gene analysis was done according to the method described previously (3). As a result, a non-overlapping library of 80 *Methylobacterium* strains was constructed.

The library was used to screen the best strain that can promote barley growth. In the first screening, sterilized barley seeds were placed on solidified soil extract solution, and bacterial suspensions were applied. The strains that showed growth promotion were selected, and this screening was done thrice for 80 strains. Finally, 8 selected strains were subjected to pot growth experiments in a greenhouse.

### 3.0 Results and Discussion

Among the 80 strains selected by MALDI-TOF analysis, up to 20 strains seemed to be novel species within the genus *Methylobacterium*. Thus, our high-throughput strategy can be successfully applied to screen novel species within the library. This method is very powerful for taxonomical study, within any library of microorganisms, including yeasts, and fungus. The novel species are currently being investigated for new species description.

In this study, the relation between isolation source and the isolates was also analyzed. The isolates belonging to *M. radiotolerance* and *M. extoquens* group were ubiquitously found in many plant species. The isolates derived from moss samples appeared to be quite unique, and most of the novel isolates were indeed from moss samples.

To examine if the isolates selected by our screening promote plant growth, 8 strains were applied to barley seeds. Effect of these strains on barley growth were measured. The result showed that total plant mass and grain yield were not improved by the application of these isolates. However, it was observed that a significant decrease in grain number and an increase in 100 seeds weight. This result implicates that although plant mass is not largely affected, application of our isolates can possibly alter plant growth, particularly in the formation of reproductive organs. Based on these observations, it was inferred that barley grain quality can be improved by the application of some isolates identified in this study.

## References

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