

論文の内容の要約

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学位論文名	<i>Methylobacterium</i> species associated with rice in Vietnam: Isolation and characterization of the new <i>Methylobacterium</i> strains causing leaf bleaching disease in rice plants

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INTRODUCTION

Plants live in complex environments where they interact with multiple detrimental or beneficial microorganisms. Therefore, the growth of plants depends on the associated microorganisms in the environment and thus the healthy and productive crop production requires a proper assembly of microbiota. The genus *Methylobacterium* is composed of pink-pigmented facultative methylotrophs (PPFMs) that belong to the class α -Proteobacteria. *Methylobacterium* species are known as persistent colonizers of the rice plant that enhance the growth and protect from potential pathogens, thus contributing to the healthy growth and productivity of the rice. However, the diversity and potential roles of *Methylobacterium* species on rice plants have not been investigated in Vietnam, one of the top rice-producing countries in the world.

RESEARCH OBJECTIVE

1. Characterize and report the bleaching symptom as a novel rice disease caused by pathogenic *Methylobacterium* strains isolated from Mekong delta, Vietnam.
2. Elucidate the mechanism underlying the bleaching symptom and propose the method to detect and prevent pathogenic *Methylobacterium* causing bleaching symptom in rice plants from paddy field.

EXPERIMENTS AND RESULTS

1. Isolation, characterization and identification of pathogens causing rice bleaching symptom

The bleaching symptom of rice plants appeared around 15-20 days after seed sowing and the symptomatic plants showed the white colour of leaves and drastically delayed growth leading to death in some cases. To isolate the causal agent, rice plants were collected from three provinces in the Mekong delta, then cultured onto selective medium and incubated at 28° C for 8 days. The pink well-isolated colonies were sub-cultured and inoculated to rice seeds for testing the induction of bleaching symptom. Seven among thirty pink isolates were chosen based on the results of inoculation test and analyzed further. All these seven isolates were pink pigmented, Gram-negative, rod-shaped and strictly aerobic. The isolates were analysed for their bio-chemical characteristics using the API 20 NE – biochemical identification system (BioMérieux). The 16S rRNA gene and *atpD* gene sequencing revealed that these seven isolates belonged to the *Methylobacterium indicum*.

2. Determine the compounds produced from the pathogenic strain VL1 that related to bleaching activity

Pathogenic *Methylobacterium* strains were cultured in liquid medium at 28° C for 7 days. The culture filtrates were obtained by centrifugation and filtration through Millipore Bacteriological Filters (0.22 um), then treated with rice seedlings. All the culture filtrates of pathogenic *Methylobacterium* strains caused the bleaching symptom on rice seedlings. No symptom of rice seedlings was observed in ¼ MS medium and in the presence of culture filtrates of *Methylobacterium indicum* strain SE 2.11^T, which is non-pathogenic strains.. The severity of the bleaching symptom depended on the concentration of culture filtrate. Further experiments will be conducted to determine the physical and biological characteristics of these compounds.

3. Explore the genes in the pathogenic strain VL1 that related to leaching activity

Whole genome sequencing revealed that *M. indicum* strain VL1 contains one chromosome and 6 plasmids with various unknown function genes. The comparison between VL1 and SE2.11 that is the non-pathogenic closest strain to VL1 showed that they are similar in chromosome but drastically different in plasmid sequences, suggesting that the genes related to bleaching symptom locate on plasmids of VL1. The

FUTURE PLANS

1. Investigate the physical and biological characteristics of culture filtrate from pathogenic strain VL1 therefore isolate and identify the responsible compounds that cause bleaching activity.
2. Identify the genes responsible for the bleaching symptom causing by pathogenic strain VL1

REFERENCES

Manuella et al. 2015. Biotechnological and Agronomic Potential of Endophytic Pink-Pigmented Methylophilic *Methylobacterium* spp. Biomed Research International. Volume 2015.