主　論　文　の　要　約

論文題目

Occurrence, distribution, genetic variability and biological properties of bacterial rice pathogens in Cambodia

カンボジアにおけるイネ病原細菌の発生・分布・遺伝的多様性および生物学的特性

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Research backgrounds

Rice (*Oryza sativa* L.) is one of Cambodia's most important field crops, being the staple food for the population, and is also a vital export commodity. Along with this demand, Cambodia produced around 7.9 million tonnes of paddy rice in the wet season. However, a great portion of Cambodian farmers' income is at risk because of possible pest and disease outbreaks. In 2016, Cambodian MAFF reported that rice

production is reduced by around 20-30% due to several factors such as pests, climate change, and soil degradation. To control these problems, farmers apply different management measures that include misuse of pesticides because of weak enforcement of current regulations and alternative pest management techniques. As pest management in Cambodia is mainly to promote best practices and is at the developing stage, the current key challenges are to gather practical solutions and to develop strategies to promote this further. Particularly for the disease management in rice, only some diseases such as rice blast are recognized in Cambodia, and this is why the basic disease survey for major rice pathogens is highly desired. In this study, rice bacterial pathogens were targeted and the surveys were conducted in the country. In addition, the genetic and biological properties of targeted bacteria were investigated.

1) The bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae*.

The yield and quality of paddy rice are affected by biotic and abiotic stresses, and one major threat is bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). In Asian countries such as China, Vietnam, Indonesia, India, and the Philippines, BLB became one of the most severe and widespread diseases, especially in the irrigated and rained lowland ecosystems. In general, yield loss is around 20-30% and may sometimes go up to 80% under a severe epidemic. The use of resistant rice varieties is the most promising approach to control the disease. Although the threat of Xoo is recognized, no fundamental information on the disease and pathogen is accumulated in Cambodia.

This study aimed to understand Xoo populations and identify the rice resistance genes effective against Xoo isolates in Cambodia. A total of 145 bacterial samples from five provinces were subjected to genotyping and pathotyping. Single nucleotide polymorphism analysis revealed that 53.75% of tested bacteria were known genotypes (population ID 3, 4, 8, 10, 13), but around half of the isolates were unknown populations, suggesting a presence of unique indigenous Xoo genotype(s) in Cambodia.

Selected 16 bacterial isolates were inoculated to 23 near-isogenic rice lines that carry single (10 plants) or multiple resistant genes (13 plants), and a local variety, Sen Pidor. Based on the lesion length, 16 Xoo isolates were classified into seven pathotypes (A to G) that are further grouped into four by Fisher's Least Significant Difference test: Group 1, pathotypes A and G (12.5%); group 2, pathotypes B and F (18.75%); group 3, pathotypes C and E (18.75%); group 4, pathotype D (50%). All Xoo groups were virulent on the rice lines possessing single resistant genes (*Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa11*, *xa13*, *Xa14*, *Xa21*, *Xa23*, and *Xa27*) with similar lesion lengths on the susceptible variety IR24 (*Xa18*). However, combinations of resistant genes generally controlled Xoo isolates, if not all, and particularly, *Xa4+xa5+Xa21* and *Xa4+Xa7+Xa21* resulted in moderate resistant - resistant to all tested Xoo isolates. The local variety showed a moderate level of Xoo-resistance but was susceptible to four bacterial isolates tested. The obtained information can be taken into account for a BLB-resistant rice breeding program in Cambodia.

2) Rice orange leaf disease pathogen, Rice orange leaf phytoplasma.

Phytoplasma is unculturable bacteria that lacks cell-wall and genes for many physiological processes, thus known as an obligate parasitic pathogen of over 1,000 species of plants. In rice, two phytoplasma-associated diseases are recognized: rice yellow dwarf disease and rice orange leaf disease. Recently, rice orange leaf disease has been reemerged in several Asian countries. The causal pathogen, Rice orange leaf phytoplasma (ROLP), belongs to the "*Candidatus* Phytoplasma *asteris*" 16SrI-B subgroup. The ecological and biological characteristics of ROLP are largely unknown because the disease previously had not widely caused serious problems, leading to a low accumulation of research data. However, in the past decade, the disease became a threat to rice production, particularly in South China and India; it has also been recognized in Thailand, and rice plants showing orange leaf discoloration have become ubiquitous in paddies in the Philippines and Cambodia. Therefore, this study conducted a series of field surveys in two provinces of the Philippines, two provinces of Vietnam, and five provinces of Cambodia. As a result, most suspected ROLP-infecting plants were positive by nested PCR detection; for example, in the total of 98 symptomatic rice plants collected from two provinces of the Philippines, 82% (Laguna) and 95% (Mindanao) were ROLP-positive. These plants showed more varying symptoms than previously reported. A phylogenetic analysis of 16S rRNA genes of ROLP isolates revealed that isolates in Thailand were diverse, and those in India and Cambodia were

fallen in the cluster of Thai isolates, suggesting their potential origins. In contrast, those in China, the Philippines, and Vietnam were almost identical and formed an independent group. This result implies a recent expansion event of a single ROLP population into these countries.

ROLP is solely transmitted by the zigzag-striped leafhopper (*Recilia dorsalis* Motchulsky) and the green leafhopper (*Nephotettix cincticeps* Uhler) (Hemiptera Cicadellidae). However, the vector insect *R. dorsalis* was scarcely present, while *N. cincticeps* was not observed in the paddies in the Philippines and Cambodia. Instead, green paddy leafhopper, *N. virescens* Distant (Hemiptera: Cicadellidae), was commonly observed in the paddies throughout all rice growing stages and different cropping seasons. Thus, the ability of *N. virescens* to transmit ROLP was thoroughly investigated. Newly emerged adult *N. virescens*, which fed on ROLD-source rice plants, were used to inoculate a susceptible rice seedling and were serially transferred into a new healthy seedling. The resultant positive transmission rates varied from 5.1% to 17.8%. The transmission ability of the insects was generally decreased over time. These findings suggest that *N. virescens* is an alternative vector of ROLP in the Philippines and Cambodia.

The ROLP-infected rice exhibits clear orange to yellowish leaf discoloration and severe stunting in seedlings, resembling symptoms caused by rice tungro viruses. Serological and genetic detection of tungro viruses suggested mostly no virus infection in the tested rice plants. Moreover, fluorescent and scanning electron microscopic observations revealed the intensive accumulations of the phytoplasma in phloem

tissues. The massive accumulation of storage starch in vascular bundle sheath and parenchyma suggested a correlation between the symptoms and the plant's energy conversion defectiveness. Altogether, this study illustrated the genetic variability of global ROLP isolates and the pathogen's biological impact on rice tissue. Moreover, the study highlighted the increasing importance of ROLD-reemergence in Southeast and East Asia. It proved the need to manage *R. dorsalis, N. virescens*, and *N. cincticeps* carefully.

Conclusions.

Overall, this study recorded the occurrence of the bacterial leaf blight disease and the rice orange leaf disease for the first time and revealed that these diseases are widespread in Cambodia with unexpectedly high genetic diversity. Along with the global mobility of people and foods or any materials, plant pathogens may accidentally contaminate and expand worldwide despite international plant quarantine services. In this sense, intensive field surveys and thorough pathogen characterizations in this study provide important information to develop the future rice protection scheme in Cambodia: identifying potentially effective rice Xoo-resistant genes and finding the ROLP-transmitting insects in the country. As rice is facing many types of biological stresses, including rodents, insects, fungi, bacteria, and viruses, more systematic surveys on rice pests and conventional disease diagnosis services in the country should be developed.