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学位論文内容の要旨

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学位論文題名

Function of regulatory gene *PoLAE2* on appressorium formation in rice blast fungus *Pyricularia oryzae*

(イネいもち病菌の包括的制御遺伝子*PoLAE2*の 付着器形成における役割)

Pyricularia oryzae caused rice blast disease is a pivotal plant-pathogenic fungi that threaten global rice production in many decades until present. This problem affects rice yields loss and world economics annually. The infection of rice blast disease initiates when fungal conidia germinate on the host surface and germ tube recognizes physical cues. Then surface signals by G-protein couple receptors activate adenylase cyclase through heterotrimeric G-proteins to generate secondary messenger cyclic AMP (cAMP) and result in activation of cAMP/PKA signaling leading the fungus develop a specialized infection structure called appressorium. *laeA* is a regulatory gene that conserved in various species of pathogenic ascomycetes fungi including *P. oryzae*. It has been studied extensively in secondary metabolism, morphological development and virulence. However, the role of *laeA* homolog (*PoLAE2*) in *P. oryzae* on appressorium formation has not been clear. In this research, *PoLAEA2* deletion mutant and its complemented strains were constructed and function of *PoLAEA2* on appressorium formation especially on cAMP signal transduction pathway was investigated.

1. Function of *PoLAE2* on appressorium formation and pathogenicity

PoLAE2 deletion mutant (Ina86-137Δ*lig4*Δ*lae2*) and its complementation strains (T9 and T11) were constructed. Onion epidermis assay was performed to investigate appressorium formation and the result showed that wild type (Ina86-137) and complementation strains (T9 and T11) exhibited high percentage of appressorium formation in killed onion epidermis. On the other hand, *PoLAE2* deletion mutant (Ina86-137Δ*lig4*Δ*lae2*) showed decrease in appressorium formation. Spraying inoculation assay on rice leaves showed that all fungal strains can cause the lesions on leaves. In addition, inoculation of fungal conidia onto intact rice sheath showed that all strains formed

appressoria on rice tissue. These results indicate that *PoLAE2* is required for appressorium formation on non-host suface, whereas it might not affected fungal pathogenicity on the rice plant.

2. Role of *PoLAE2* on cAMP signal transduction for appressorium formation

In order to determine the role of *PoLAE2* on cAMP signal transduction, exogenous cAMP was added to fungal conidia suspension and inoculated on onion epidermis to examine appressorium formation. The appressorium formation of *PoLAE2* deletion mutant was recovered. Similar result was observed when 3-isobutyl-1-methylxanthine, which is potent inhibitor for phosphodiesterase, was applied. These results indicated that *PoLAE2* deletion affected cAMP signaling pathway, by decreasing the intracellular cAMP concentration. To examine function of PoLAE2 on intracellular cAMP concentration, cAMP was extracted from liquid-cultured mycelia of each fungal strain. High-performance liquid chromatography (HPLC) was used to analyzed intracellular cAMP level. The result showed that intracellular cAMP concentration in *PoLAE2* deletion mutant significantly lower than wild type, complementation strains no.9 and no.11. These results confirmed the relationship of PoLAE2 and intracellular cAMP level in rice blast fungus.

3. Transcription analysis of genes related to cAMP level in the *PoLAE2* deletion mutant

Relationship between *PoLAE2* and genes that balance cAMP level in cAMP signal transduction, *MAC1* (adenylate cyclase) and *PDEH* (phosphodiesterase) was investigated. RNA of each fungal strain was extracted and reverse transcription-polymerase chain reaction (RT-PCR) was used for the transcription analysis. Results of RT-PCR revealed that there are no significant difference in transcription level of these genes in wild type, *PoLAE2* deletion mutant and complementation strains. These results suggest that *PoLAE2* might not concerned directly with regulation of *MAC1* and *PDEH* at transcription level.

This research demonstrated that the regulartory gene, *PoLAE2* is essential for appressorium formation on non-host surface. Moreover, PoLAE2 also concern with cAMP signaling pathway by regulates intracellular cAMP level. It is also suggested that at least low concentration of cAMP level is required appressorium formation on any surface, and PoLAE2 might compensate appropriate cAMP level to support the fungus to form appressorium on non-host surface. This is the first report to reveal the relationship between *PoLAE2* and cAMP signaling in filamentous fungi.