学位論文内容の要旨

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学位論文題名

Mitochondrial visualization in rice blast fungus and its application to 3D observation, quantification, and distribution analysis

（イネいもち病菌のミトコンドリアの可視化とその３次元観察，定量及び分配解析への応用）

Pyricularia oryzae is a multi-host pathogenic fungus that causes rice blast disease, one of the most serious threats to our global food security. Recently, emergence and spread of QoI fungicide resistance is a serious problem in many plant pathogens including *P. oryzae*. This QoI fungicide targeting cytochrome b in mitochondria and affect respiratory system to kill the pathogen. QoI resistance is caused by a base substitution in cytb gene in mitochondria DNA. Conidia of *P. oryzae* are important for the spread of the pathogen. Therefore, for the elucidation of the rapid spread of QoI resistance in the natural population of *P. oryzae*, it is necessary to analyze the dynamics of mitochondria, especially during conidia formation. In this study, visualization of mitochondria by GFP was attempted. In addition, the technique was utilized for three-dimensional observation, quantification, and distribution to conidia.

1. Visualizing mitochondria in *Pyricularia oryzae* during conidia development with Citrate Synthase-GFP fusion protein

In order to visualize mitochondria, *P. oryzae* was transformed using citrate synthase (CitA)-GFP fusion protein. The vector was constructed by fusing GFP gene to N-terminal part of cintrate synthase, which produced in cytosol, and then transferred to mitochondria. The localization of GFP in mitochondria was confirmed by colocalization of GFP with mitochondria specific MitoTracker Red under fluorescence microscopy. Then appropriate culture method for the observation of conidia development was investigated using the transformant. It is revealed that after 36 hours of incubation on oatmeal agar, conidia with one cell, two cells and three cells were harvested, and categorized them as baby conidia, young conidia, and matured conidia, respectively. 3D visualization of the conidial

mitochondria in the transformant was performed with laser-scanning confocal microscopy and computer analysis with Las X software. The result indicated that mitochondria could be distributed into baby conidia as a dot and develop to a network as aging of conidia.

2. Quantification of Conidial Mitochondrial Volume using software MitoGraph

MitoGraph is an automated image processing software dedicated to calculating the volume of three-dimensional organelles and intracellular structures in live cells. In this study, this software was used to measure mitochondrial amount of randomly-collected baby, young until matured conidia as 3 dimensional-object, and characterize further the mitochondria development. Analysis of the conidial mitochondrial volume against conidial length showed that increase of mitochondrial volume did not correlated with the cellular size, different from the case of *Saccharomyces cerevisiae*. This observation indicated that cellular growth and mitochondrial growth were not synchronized. However, there remained a strong possibility of the the result was affected by the timing of conidia harvest. Continuous observation of the conidial development from conidiophore with the GFP-stained mitochondria was required for further characterization of mitochondria dynamics in the developing conidia.

3. Visualization of mitochondrial dynamics during conidia formation using slide culture-confocal microscopy

The slide culture method for conidia formation on a filter paper was established. After incubation for four days, conidia formation could be observed simultaneously under fluorescence microscopy. This observation revealed that duration of conidia development from conidiophore was about 2.5 hours. By further observation under confocal microscopy with time-lapse recording, this method will give the opportunity to see mitochondrial development from baby conidia until matured conidia and measure the mitochondrial volume for their every stage.

Taken together, this thesis established the observation method of mitochondria in living cells in *P. oryzae*. In addition, it also revealed that basic mitochondria dynamics in hyphae and conidia of the rice blast fungus. These knowledges should contribute to further understanding of mitochondria distribution and inheritance in *P. oryzae*, which is an important key to control the spread of QoI fungicide resistance.