Study on Mechanism of Elaborated Hypoxic Condition in Cordycepin Biosynthesis of Cordyceps militaris

（課程博士・様式７） (Doctoral qualification by coursework, Form 7)

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：Study on Mechanism of Elaborated Hypoxic Condition in Cordycepin Biosynthesis of *Cordyceps militaris*
（*Cordyceps militaris* のコルジセピン生合成における低酸素状態メカニズムに関する研究）

論文要旨：An entomopathogenic fungus, *Cordyceps militaris* is an insect infecting fungus, controlling their behavior and mummifying its insect body. *C. militaris* produces many bioactive compounds, including amino acids, polysaccharides, fatty acids, nucleosides, vitamins, etc. Among them, cordycepin is the most popular compound provided only by this species. The analog structure of cordycepin can alter the DNA/RNA synthesis by inhibiting the polyA polymerase activity process of the mRNA. Its activity makes it a unique candidate for the anticancer agent with the high global market demands and makes it the most expensive drug in the world. There are many difficulties faced while mimicking the production on the laboratory scale. Moreover, the quality of cordycepin based on laboratory-based production is low compare to the natural one. Our previous study revealed that liquid static culture conditions induced the high production level of cordycepin compared to the submerged culture. The RNA expression level compared between both states along the cordycepin biosynthesis stages. The fundamental hypothesis of the results study suggested that hypoxic conditions triggering high cordycepin production. Further compared the transcriptome level between the aerial mycelium and submerged mycelium. The assumption is that submerged mycelium facing the hypoxic stress condition and the main contributor of the cordycepin biosynthesis. It is worth mentioning that the unique morphology development of the small cake-like floating mycelium observed during the early culture periods. Later, these little pieces were progressively assembled into the thick layer mycelium and covering the surface media, completely. The transcriptome results showed that genes in the submerged mycelium elaborated on hypoxic response conditions were more upregulated. Interestingly, the heme biosynthesis activity was upregulated before the exponential phases of cordycepin biosynthesis. It conceivable that the

activation of the cordycepin biosynthesis cluster genes depended on the iron sequestrated from the heme metabolism. To clarify that, CRISPR/Cas9 endonuclease was implemented in *C. militaris.* Since this is new methods, the development strategy of transformation, growth medium, and selection marker was needed. Here, we successfully developed the blastospore transformation system and found the appropriate growth medium. Two kinds of a selection marker, an auxotroph pyrG mutant and a yellow color phenotype of the neurosporaxanthin pathway, were achieved.