

学 位 論 文 要 旨	
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題 目	Studies on citrate transporters in the white koji fungus, <i>Aspergillus luchuensis</i> mut. <i>kawachii</i>
<p>A white koji fungus, <i>Aspergillus luchuensis</i> mut <i>kawachii</i> (<i>A. kawachii</i>) is used for production of shochu, a Japanese traditional distilled spirit. <i>A. kawachii</i> was isolated as an albino mutant from the black koji fungus, <i>Aspergillus luchuensis</i>, and they produce a large amount of citric acid. This feature is important because shochu is mainly produced in relatively warm areas of Japan, such as Kyushu.</p> <p>In this study, firstly, we found that <i>A. kawachii</i> produces black colored conidia by complementation of the wild type <i>pksP</i> gene, which encodes a polyketide synthase involved in the melanin biosynthesis. The <i>pksP</i> complementation did not affect the colony size or conidial surface structure in <i>A. kawachii</i>, but did caused an increase in the number of conidia per colony area. Moreover, the conidia of <i>pksP</i> complementation and overexpression strains showed higher resistance against ultraviolet light, hydrogen peroxide, and ion beams than that of the control strain.</p> <p>Next, we characterized citrate transporters CtpA and YhmA in <i>A. kawachii</i>. In biochemical analysis using purified CtpA and YhmA, we identified their citrate transport activity. Disruption of <i>ctpA</i> and <i>yhmA</i> caused deficient hyphal growth and conidium formation with reduced mycelial weight-normalized citrate production. Because we could not obtain double knockout strain, we constructed conditional double knockout strain using the Tet-On promoter system. Knockdown of <i>ctpA</i> and <i>yhmA</i> resulted in a severe growth defect on minimal medium with significantly reduced acetyl-CoA levels; however, we subsequently found that the severe growth defect was relieved by addition of acetate or lysine, which could remedy the acetyl-CoA level. These results indicate that CtpA and YhmA are mitochondrial citrate transporters involved in citric acid production and that transport of citrate from mitochondria to the cytosol plays an important role in acetyl-CoA biogenesis in <i>A. kawachii</i>.</p> <p>Finally, we characterized the role of putative methyltransferase LaeA for citric acid hyperproduction in <i>A. kawachii</i>. The $\Delta laeA$ strain exhibited a significant reduction in citric acid productivity. Cap-analysis gene expression (CAGE) revealed that <i>laeA</i> is required for the gene expression of a putative citrate exporter encoding <i>cexA</i>. The deficit in the productivity of citric acid by the $\Delta laeA$ strain was rescued by the overexpression of <i>cexA</i>. In addition, ChIP-qPCR analysis indicated that LaeA regulates the expression of <i>cexA</i> via controls the euchromatin/heterochromatin ratio. These results indicate that LaeA is involved in citric acid production through epigenetic regulation of <i>cexA</i> in <i>A. kawachii</i>.</p>	